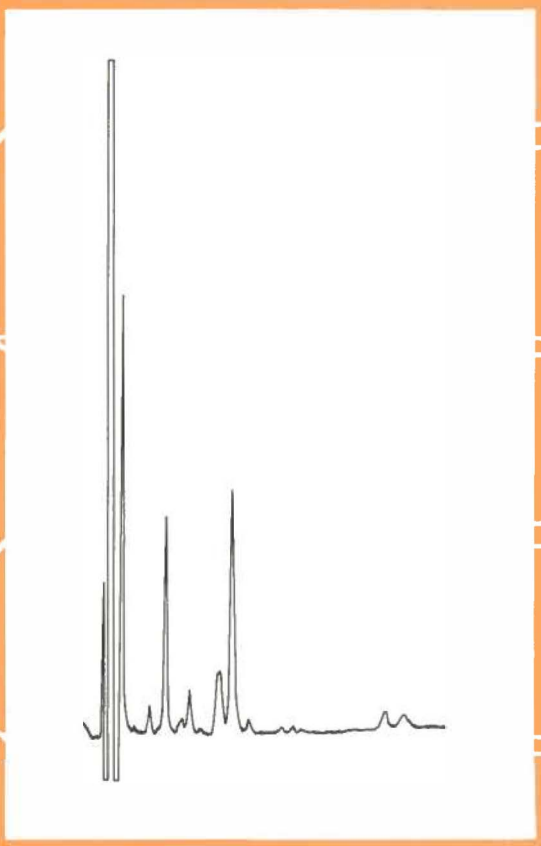
Long chain omega-3 polyunsaturated fatty acids in rheumatoid arthritis

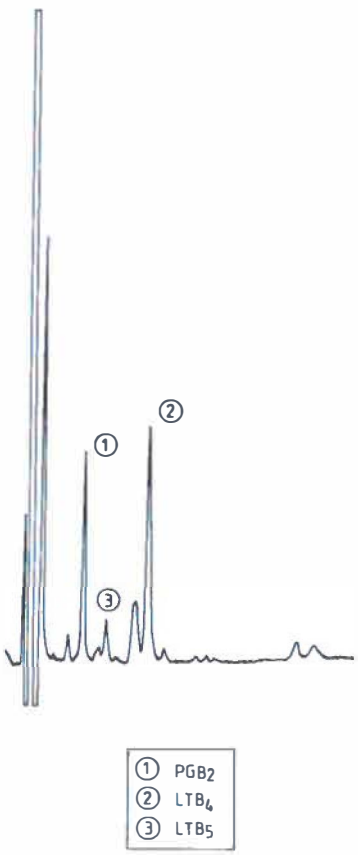
Tulleken, Jacob Ernestus

LONG CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS IN RHEUMATOID ARTHRITIS



J.E. TULLEKEN

LONG CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS IN RHEUMATOID ARTHRITIS



To the cover:
The drawing in the middle represents the reversed-phase high-performance liquid chromatogram of leukotriene B4 and leukotriene B5 produced by human neutrophils. PGB2 was added as an internal standard.

Stellingen behorende bij het proefschrift van
J.E. Tulleken

LONG CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS IN RHEUMATOID ARTHRITIS

Groningen, 8 mei 1991

---

1. Het gebruik van visolie dient als aanvulling op de medicamenteuze behandeling van reumatoïde artritis te worden beschouwd.

2. De remmende invloed van omega-3 vetzuren op de interleukine-1 produktie wordt niet weerspiegeld door veranderingen in de gemeten acute fase respons bij patiënten met reumatoïde artritis.

3. Vitamine E is van secundair belang voor het bereiken van het gunstige effect van diëtaire visolie suppletie op gewrichtsklachten.

4. Visolie vetzuren dienen bij voorkeur in hun natuurlijke 'triglyceride' vorm te worden aangeboden.

5. Door de recente ontwikkeling van selectieve lipoxygenase remmers en leukotriëne receptor antagonisten zal het inzicht in de rol van leukotriënen in ontstekingsprocessen worden vergroot.

6. Je bent wat je eet.

7. De patiënt-vriendelijke verpakkingen van reuma geneesmiddelen zijn vaak kind-onvriendelijk.

8. In tegenstelling tot de bestrijding van natuurlijke virussen is de bestrijding van computervirussen simpel: je hoeft alleen maar iemand te vinden die slimmer is dan de ontwerper.

9. De gemeten concentratie van biologische mediatoren in lichaams-compartimenten is niet zonder meer bepalend voor hun pathofysiologische betekenis.

10. In het kader van 'zorg op maat' vervult de reuma-consulent(e) met specifiek verpleegkundige kennis en ervaring een belangrijke aanvullende rol in de behandeling en begeleiding van patiënten met chronisch invaliderende reumatische aandoeningen.

11. Touroperators doen er verstandig aan hun financiële reserves te beleggen in Nederlandse pret- en bungalowparken.

12. De visolie wordt duur betaald.

RIJKSUNIVERSITEIT GRONINGEN

LONG CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS IN RHEUMATOID ARTHRITIS

Proefschrift

ter verkrijging van het doctoraat in de
Geneeskunde
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus Dr. L.J. Engels
in het openbaar te verdedigen op
woensdag 8 mei 1991
des namiddags te 14.45 uur precies

door

JACOB ERNESTUS TULLEKEN

Geboren op 5 september 1959
te Winschoten

Promotor	:	Prof. Dr. M.H. van Rijswijk
Referenten	:	Dr. P.C. Limburg Dr. F.A.J. Muskiet

Promotiecommissie	:	Prof. Dr. J.K. van der Korst Prof. Dr. W.D. Reitsma Prof. Dr. W. van der Slik

This study was financially supported by grants 86/CR/59, 87/CR/41, 88/CR/057, 89/CR/210 from the Dutch Rheumatism Foundation.

The oil supplements were kindly provided by Aerofako bv, PO Box 1201, 3800 BE Amersfoort, The Netherlands.

The printing costs of this thesis were financially supported by: Aerofako, Bencard (afdeling van SmithKline Beecham Farma), Ciba-Geigy, Dagra-Pharma, Roche Nederland, Pfizer.

Aan: Carla
Leonie, Bob
mijn ouders

krips repro meppel

CONTENTS

Voorwoord		1
Chapter 1	An introduction to rheumatoid arthritis, dietary fish oil and eicosanoid synthesis	3
Chapter 2	Effects of fish oil supplementation in rheumatoid arthritis *Ann Rheum Dis 1990;49:76-80*	25
Chapter 2a	Omega-3 polyunsaturated fatty acids, interleukin-1 and tumor necrosis factor *N Engl J Med 1989;1:55-56 (Letter)*	35
Chapter 3	Vitamin E status during dietary fish oil supplementation in rheumatoid arthritis *Arthritis Rheum 1990;33:1416-1419*	37
Chapter 4	Non steroidal antiinflammatory drug demand during fish oil treatment in rheumatoid arthritis *Submitted*	43
Chapter 5	A comparison between the effects of fish oil ethyl esters and fish oil triglycerides in rheumatoid arthritis *Submitted*	51
Chapter 6	The effect of dietary energy percentage of fat and its P/S ratio on incorporation of n-3 PUFA and leukotriene production during dietary fish oil supplementation in healthy volunteers *Submitted*	63
Chapter 7	Effects of long chain omega-3 polyunsaturated fatty acids in rheumatoid arthritis *Advances in rheumatology and inflammation (in press)*	75
Summary		85
Samenvatting		89

VOORWOORD

Het onderzoek, beschreven in dit proefschrift, werd verricht op de afdeling Reumatologie van de Kliniek voor Inwendige Ziekten (Hoofd: Prof. Dr. G.K. van der Hem) van het Academisch Ziekenhuis te Groningen. Het onderzoek werd mogelijk gemaakt door een subsidie van het Nationaal Reumafonds. Het tot stand komen van dit proefschrift is te danken aan een groot aantal personen. In de eerste plaats ben ik de patiënten dank verschuldigd. Zonder hun bereidwillige medewerking was het onmogelijk geweest de gegevens op deze wijze te verzamelen.

Prof. dr. M. H. van Rijswijk, mijn promotor, initieerde het onderzoek en was verantwoordelijk voor vele suggesties en verbeteringen. Beste Martin, ik ben je zeer dankbaar voor de steun en de begeleiding in vele opzichten, waardoor dit proefschrift op een efficiënte wijze kon worden voltooid.

Dr P.C. Limburg dank ik voor zijn grote bijdrage aan het onderzoek. Beste Piet, jij wist als geen ander mij weer op 'koers' te krijgen als ik weer eens 'stuurloos' was. De plezierige en persoonlijke manier waarop jij mij steeds hebt begeleid, heb ik ten zeerste gewaardeerd.

Dr F.A.J. Muskiet was als vetdeskundige onmisbaar. Beste Frits, je aanstekelijk enthousiasme en wijze lessen op vetgebied hebben hun uitwerking niet gemist. Dank je voor de stimulerende samenwerking.

Met veel plezier denk ik terug aan de bijzonder prettige samenwerking met de andere leden van het 'visolie-team'. Wieke Wassenaar (research-verpleegkundige) wil ik hartelijk danken voor de goede persoonlijke begeleiding van de patiënten en voor het voeren van een perfecte administratie. De diëtisten Marga Strubbe-Verloop, Immie Boomgaardt en Sietske Huitema hebben op uitstekende wijze de patiënten begeleid bij het 'vet-constante' dieet. Van alle medewerkers van het reumatologie laboratorium dank ik in het bijzonder Karin Kazemier, voor haar inzet en de accuratesse waarmee ze diverse laboratorium bepalingen heeft opgezet, uitgevoerd en verder geperfectioneerd. Drs H. (Hille) van der Tempel ben ik erkentelijk voor zijn belangrijke rol in de beginfase van het onderzoek.

Van het Centraal Klinisch Chemisch Laboratorium (Prof Dr. W. van der Slik) verrichtten de medewerkers, Ingrid Martini, Gert Meiborg, Martine van der Molen, Henk Rutgers, Herman Velvis en Inge Venekamp-Holsema met veel enthousiasme de vetzuur en vitamine E bepalingen.

Drs L. (Lodewijk) R. Berkenbosch van de firma Aerofako zorgde immer voor een tijdige levering van de visolie terwijl drs. M. Laseur, apotheker van het AZG, de uitvulling van de capsules organiseerde.

Dr J. (Jaap) J. van Doormaal dank ik voor de stimulerende discussies omtrent vetzuurgebied. Dr W (Wim) J. Sluiter en dr V. Fidler waren behulpzaam bij de statistische analyses.

De collegae van de afdeling Reumatologie (drs M. (Miek) A. van Leeuwen, drs B. (Bouke) P.C. Hazenberg, dr. E.J. (Evert-Jan) Ter Borg, dr. R.L.F. (Bob) Nienhuis en drs M. (Marianne) A.C. Vierboom ben ik erkentelijk voor hun goede adviezen en de prettige werksfeer.

Janny Wever (secretaresse) heeft in belangrijke mate bijgedragen aan de lay-out van dit proefschrift en was evenals Anneke Boer (secretaresse) en Adrien Schuttenbeld-Remijn (regas) behulpzaam waar nodig.

Jacob Pleiter verrichtte het foto-werk.

Bert Schaalma, Els Muskiet-Stuut, en drs A. (Anita) Verhoeven assisteerden bij het verzamelen van literatuur gegevens.

Jan Uni en Dick van Gennep zorgden voor de koffie en gaven adviezen omtrent vele andere zaken die geheel los stonden van het onderzoek.

Verder wil ik alle collegae, vrienden, familie en kennissen die op enigerlei wijze hebben bijgedragen aan de tot standkoming van dit proefschrift hartelijk bedanken.

Bovenal dank ik mjn lieve vrouw Carla die thuis de regie verzorgde.
En Leonie en Bob....., papa zal nu eindelijk voor *jullie* een tekening op de computer gaan maken.

Chapter 1

AN INTRODUCTION TO RHEUMATOID ARTHRITIS, DIETARY FISH OIL AND EICOSANOID SYNTHESIS

1. RHEUMATOID ARTHRITIS

Definition and classification

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology, mainly characterized by joint inflammation leading to destruction and deformity. Although arthritis is the predominant clinical feature, RA is to be considered as a systemic disease since it can have a wide range of non articular manifestations. The prevalence of the disease increases with advancing age up to the seventh decade, and varies between 0.3% and 1.5% of the population in various parts of the world[1]. The overall women to men sex ratio is about 3:1. Classification criteria for RA were developed by a committee of the American Rheumatism Association in 1956[2]. These criteria were formulated from the experiences of the five committee members, epidemiologic survey, and 332 cases provided by physicians in the United States of America and Canada. Eleven criteria with 19 exclusions were proposed. 'Definite' RA required at least 5 criteria and 'probable' RA required at least 3 criteria. In 1958 the category 'classic' RA was added to describe patients who fulfilled 7 of the original 11 criteria[3]. The 1987 revised RA criteria imply 7 criteria (Table 1). Items 1 through 4 must have been present for at least 6 weeks to meet the criteria for RA[4].

Table 1. The 1987 revised criteria for the classification of rheumatoid arthritis

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement.
2. Arthritis of 3 or more joints	At least 3 joint areas simultaneously have had soft tissue joint areas swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints.
3. Arthritis of hand joints	At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint.
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry).
5. Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxtaarticular regions, observed by a physician.
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by a method for which the result has been positive in <5% of normal control subjects.
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify).

Symptoms and signs:

In characteristic cases the joint involvement is bilateral and symmetrical, but unilateral involvement may also occur. Pain and stiffness, leading to impaired function are the predominant symptoms. The joints are usually swollen by synovial hypertrophy and effusion whereas during active periods and exacerbations the joints are warm, and extremely tender. A few months period of fatigue, weight loss and general malaise may precede joint symptoms. However, not all patients claim symptoms prior to the

onset of joint disease. In chronic stages bony overgrowth or subluxation may give the impression of joint swelling. Persistent inflammation leads to muscle wasting and stretching or even rupture of tendons around affected joints. A typical early feature of the inflammatory process in bone is periarticular osteoporosis. In later stages, loss of cartilage, erosion and destruction of bone may occur which may ultimately lead to complete loss of function of affected joints. Although in fact any diarthrodial joint can be involved in RA, the proximal interphalangeal (PIP), the metacarpophalangeal (MCP) and the metatarsophalangeal (MTP) joints are most commonly affected. Ulnar deviation at the MCP joints and Swanneck and Boutonniere deformities may occur over months or years resulting in an additional limitation of hand function. In the knees, the patient typically complains about pain and difficulty in walking, climbing stairs and arising from chairs. Pain and swelling behind the knee may reveal a Baker's cyst, due to extension of the synovium into the popliteal space. Limitation of daily locomotion is additionally hampered by broadening of the forefeet with subluxation of the MT heads, overriding toes, fibular deviation and hallux valgus as well as local pressure sores. A major cause of concern is the cervical spine synovitis and bursitis, which may lead to atlantoaxial subluxation with compression of the spinal cord.

Systemic manifestations are common and may precede joint symptoms or even dominate the clinical picture. For instance subcutaneous rheumatoid nodules may be the presenting feature of the disease and are most frequently found at the elbow. Lymphadenopathy, splenomegaly and muscle wasting are some systemic features of the disease as well. The course of the disease may be further complicated by secondary amyloidosis, increased susceptibility for infections and side effects of drug treatment e.g. steroid induced osteoporosis.

A wide variety of different variables have been advocated for monitoring disease activity in RA. Conventional laboratory parameters include: erythrocyte sedimentation rate (ESR), rheumatoid factor titre, C-reactive protein (CRP), platelet count and haemoglobin. The levels of CRP and the ESR often increase during periods of active disease. Alterations in their levels correspond well with changes in disease activity. A majority of patients with RA have elevated titers of rheumatoid factor in their serum. Rheumatoid factors are antibodies against regions of the Fc portion of human IgG. The Latex fixation- and Waaler-Rose tests, are mostly used and mainly detect the presence of IgM-rheumatoid factor. Rheumatoid factors among the IgA and IgG classes of immunoglobulines have been found as well, using more sophisticated immunoassays[5]. The test for IgM-rheumatoid factor is positive in about 70% of the cases, usually before the second year of the disease. However, in 1-5% of normal subjects and in a number of other diseases IgM-rheumatoid factors can be detected as well. This indicates that a positive test for IgM-rheumatoid factor is not entirely specific for RA.

Soft tissue swelling and periarticular osteoporosis of affected joints are early radiographically demonstrable changes characteristic for the disease. In later periods, loss of joint space, bone destruction, development of cysts, and secondary osteoarthrosis may develop. Magnetic resonance imaging may be the best imaging technique for early detection of synovial proliferation and pannus formation, but is in general regarded to be too expensive for routine use.

Besides objective laboratory and radiographic methods for monitoring disease activity, grading and recognizing the patient's subjective pain response is used for the evaluation of the efficacy of anti-rheumatic drugs. A well known example is the Ritchie Articular index[6]. This index is based on the summation of a number of quantitative evaluations of the pain experienced by the patient when the joints are subjected to firm pressure exerted over the articular margin. In the last decade functional indices such as the Health Assessment Questionnaire (HAQ)[7] and the Arthritis Impact Measurement Scale (AIMS)[8] have been developed. Careful evaluation have proven them to be useful in determining the long term impact of RA and the effect of treatment on the quality of life of RA patients[9].

Etiology and pathophysiology

There are reasons to assume that RA is a diagnosis applied to a non homogenous population of patients whose disease is the result of a variety of exogenous or autologous trigger factors[10,11]. The earliest event in the process is assumed to be the presentation of an unknown antigen in an immuno-genetically susceptible host. Antigen presentation to T-cells occurs in the context of major histocompatibility complex class II encoded proteins. The amino acid sequence in the third hypervariable region of the ß1-chain of HLA-DR4, in particular, appears to have a strong association with susceptibility for developing RA[12]. A pathogenetic role for exogenous infectious candidates has been ascribed to e.g. Epstein Barr virus (EBV). Many patients with RA have high serum antibody titers against EBV[13], however, not in the early stages of the disease[14], suggesting a secondary role for this organism in the development of RA[10]. In animal models, collagen type II can induce arthritis. In humans however, autologous antigens including collagen type II and IgG, have been implicated in the progression rather than in the induction of RA. In a small subset of patients immunologic sensitivity to certain food elements has been identified. In such patients nutritional modification might affect disease activity[15].

The model of tissue destruction is complex and suggests involvement of both agonist and antagonist molecules in the cell-cell interactions[16]. Antigen presenting cells can be recognized by T-lymphocytes, resulting in the release of cytokines. This will e.g. lead to activation and differentiation of B-cells into antibody producing cells. Concomitantly, macrophages and synovial fibroblasts are induced to produce and secrete interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), IL-6 and IL-8. The local production of IL-1 is e.g. chemotactic for lymphocytes, neutrophils and monocytes, it stimulates endothelial cells to bind T and B lymphocytes, and it induces fibroblast proliferation. In addition, both IL-1 and TNF-alpha may induce chondrocytes and synovial fibroblasts to produce collagenases and neutral proteases. Recent studies have indicated, however, the existence of e.g. IL-1 inhibitors which can diminish the local effects of IL-1 in the joint, illustrating the complex mechanisms involved in the process of tissue destruction[16]. Binding of antibodies to antigen will lead to the formation of immune complexes and complement activation. Phagocytosis of the immune complexes by polymorphonuclear cells causes release of eicosanoids, free radicals and proteases which are directly responsible for the signs and symptoms of inflammation and tissue damage. At this stage of the disease the proliferating synovial membrane (pannus) behaves much like a localized neoplasia, invading

cartilage, subchondral bone and periarticular structures leading to irreversible damage of the joint[10].

Course and prognosis

During treatment, the course of the disease may show complete (in approximately 15% of the patients) or partial (in 25%) remissions. The remaining patients suffer from progressive disease, which faster or slower will ultimately result in physical disability[11]. A recent review of the literature identified female sex, positive rheumatoid factor, the appearance of rheumatoid nodules, and long standing elevation of ESR and CRP as predictors of disability[17].

A reduced life expectancy of RA patients has been stressed by several reports[18,19]. An average reduction of 7 years in males and of 3 years in females was reported by Vandenbroucke et al[19] in a 25 years prospective follow up of 209 patients. Mitchell et al[20] noted that 10-20% of the excess deaths appeared to be due to disease related causes, infections and to gastrointestinal complications. The numbers of death from cardiovascular disease or malignancy did not exceed those in a general population.

Treatment of rheumatoid arthritis

The main target of professional patient care is to improve the clinical outcome of the individual patient. In most cases a team of health professionals is involved in the care for the RA patient. Such a team should include a rheumatologist, surgeon, general practioner, physiotherapist, occupational therapist, pharmacist and dietician. More recently dedicated nurse specialists have been implicated in some arthritis teams as well, bridging the communication gap between the in-hospital specialist expertise and the home-care team activities. The primary care for the patient and his or her family should include the instructions concerning the possible course of the disease and its implications for daily living activities, the rationale of pharmacologic and non pharmacologic treatment as well as the facilities provided by allied health professionals.

The basic pharmacologic treatment for RA comprises two concomitant regimens, first, alleviation of symptoms, and second, suppression of the activity of the disease itself. For the alleviation of symptoms nonsteroidal antiinflammatory drugs (NSAID) are prescribed. Their analgesic capacities may help to settle the pain but do not cure the disease. It has been accepted world wide that NSAID exert their effect by inhibiting prostaglandin synthesis. However, recent work has indicated that inhibition of the cyclooxygenase pathway may not be the sole mechanism of action of NSAID[21]. For the suppression of disease activity additional treatment is instituted with so called 'disease modifying anti-rheumatic drugs' (DMARD), sometimes referred to as 'second line' or 'slow-acting-antirheumatics', such as hydroxychloroquine, gold salts, salazopyrine, d-penicillamine, azathioprine, and methotrexate. These drugs share the ability to suppress disease activity. Their effectiveness is either based on empirical data or on immunosuppressive action. Prescription of low dose prednisone is in general restricted for either bridging the lag time between institution of DMARD and the occurrence of their possible effect or for the treatment of very severe cases after complications with cytostatics. Newer drug therapies under investigation include e.g. ciclosporin[22], minocycline[23] and the combination of remittive agents. The efficacy of ciclosporin has

been demonstrated in controlled studies. However, the major issue with ciclosporin is its nephrotoxicity. Perhaps a combination of ciclosporin and fish oil may reduce impairment of renal function[24]. In general, it takes some months before the effect of DMARD becomes noticeable. Although in a number of patients clinical and laboratory improvement will occur, the ultimate effects are often incomplete and not sufficient enough to control the progressive joint destruction. In addition, the usage of DMARD is often accompanied by side reactions, which may necessitate early discontinuation of the drug. In such situations treatment will generally be changed to another DMARD. In all cases serial monitoring for drug effectiveness and for side effects with gradual dose reduction after sustained remission, is a necessary prerequisite for the successful use of DMARD. A major point of discussion is to which patient or subgroup of patients and at what stage of the disease DMARD should be applied. In a recent review Harris[10] focused on the fact that the progression of the disease to a situation of irreversible joint destruction may occur at a relatively early stage (within 2 years). Awaiting the occurrence of radiographical evidence of joint space narrowing and bone erosions may, therefore, induce an unacceptable delay of adequate treatment. There is an urgent need for the assessment of factors that enable the prediction of the course of the disease at an early stage. Whether aggressive therapy at that stage will favourably alter the course and prognosis of the disease is subject to further investigation.

The role of nutrition in RA

Assessment of the dietary history has shown deficiencies in intake of essential nutrients in patients with RA[25,26]. Comparisons were made with the 'recommended daily allowances' (RDA)[27]. The RDA are supposed to indicate the necessary intake of nutrients as an average level over a period of days or weeks to maintain good nutritional health for a general population. No knowledge is, however, available regarding the applicability of the RDA to patients with RA. It is tempting to speculate that as a consequence of chronic inflammation, preferential loss of proteins, vitamins and minerals may occur, necessitating the definition of higher RDA for RA patients. Moreover, articular dysfunction, general malaise and use of drugs that interfere with absorption[28] or metabolism of nutrients[29] do not contribute to well nourishment. The aforementioned factors are held responsible for the malnourished state that was demonstrated in some patients with severe disease[26].

Some chronically ill patients tend to adjust to the general belief that rheumatic complaints originate from food components and therefore resort to unproven dietary regimens that may place them additionally at risk for nutritional deficiencies. Panush[30] noted, however, that in most patients who claimed to have food related symptoms, no clear evidence could be obtained that elimination of a food allergen did alleviate the disease symptoms. Nevertheless, in a small subset of patients hypersensitivity to certain food constituents has been identified. In such patients nutritional modification might affect the manifestation of the disease[15,30,31]. Fasting has been found effective in suppressing joint symptoms[32,33], but a more sophisticated approach hypothesizes that alteration of the dietary fatty acid composition may be of benefit in RA patients. For instance, evening primrose oil, rich in gamma-linolenic acid, was studied by Belch et al[34]. Significant clinical improvement in combination with reduction of the usage of

NSAID occurred in some patients. Other classes of fatty acids that have been advocated to posses therapeutic potentials in RA are found in fish oil. Effects of dietary fish oil on the production of some inflammatory mediators are discussed below.

2. DIETARY FISH OIL AND EICOSANOID SYNTHESIS

Introduction

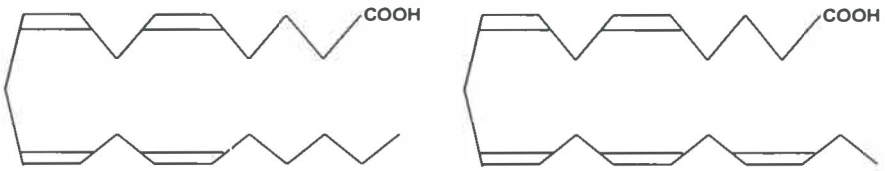
Epidemiological studies in the Upernavik district of Northwest Greenland have shown that Eskimo natives, consuming large amounts of fish and marine mammals have a low prevalence of coronary heart disease[35,36] In addition, a Dutch study demonstrated that mortality from coronary heart disease was more than 50% lower among persons consuming at least 30 g fish/day, as compared to those who did not eat fish at all[37]. Cold water fish and fish oils are rich in omega-3 polyunsaturated fatty acids with 20 carbon atoms or more. Evidence is now accumulating that consumption of omega-3 polyunsaturated fatty acids leads to profound biological actions which may be relevant to atherosclerosis and chronic inflammatory diseases. Regarding its ability to modulate eicosanoid production, fish oil supplementation has been advocated as a therapeutic modality for rheumatoid arthritis[38]. This part of the chapter will focus briefly on the influence of long chain omega-3 polyunsaturated fatty acids on eicosanoid production.

Polyunsaturated fatty acids

Triglycerides are the main sources of dietary fatty acids. Fatty acids without unsaturated bonds between their carbon atoms are designated saturated fatty acids, whereas fatty acids with one or more double bonds are named mono- (MUFA) or polyunsaturated (PUFA) fatty acids, respectively. The position of double bonds is of importance to the metabolism of the fatty acid. MUFA and PUFA can be characterized by the number of carbon atoms from the last double bond up to and including the terminal methyl group of the carbon chain. PUFA with three (omega-3; n-3 fatty acids) or six (omega-6; n-6 fatty acids) carbon atoms in that position can not be *de novo* synthesized by the human body and hence are called essential fatty acids. The 'stem' essential fatty acids are linoleic- (18:2,n-6) and alpha-linolenic (18:3,n-3) acids. These may be modified by chain elongation and desaturation. Arachidonic- (20:4,n-6) (see Figure 1) and docosahexaenoic (DHA; 22:6,n-3) acids, as a result of modification of 18:2,n-6 and 18:3,n-3, respectively, are essential in e.g. the developing fetal brain[39]. A deficiency syndrome of n-3 PUFA leading to functional changes in the retina and the visual system has been described in rhesus monkeys[40]. Long term suboptimal dietary intake of n-6 PUFA from the diet may lead to dermatitis, impaired growth, reproductive failure and kidney and liver pathology[41]. Some vegetable oils are rich in n-6 PUFA, of which 18:2,n-6 is the quantitatively most important species. Terrestrial plants contain 18:3,n-3 in their chloroplast membranes. Fish from cold water seas and refined fish oils are the richest sources of n-3 PUFA with 20 carbon atoms or more. The main members of those long chain n-3 PUFA series are eicosapentaenoic acid (EPA; 20:5,n-3) (see Figure 1) and 22:6,n-3.

The 20:4,n-6 is an important constituent of cell membrane phospholipids. Its dietary intake is usually low since there are no major sources in regular food. Cells are, therefore, largely dependent upon direct uptake of dietary 18:2,n-6 from

Figure 1 Structures of arachidonic and eicosapentaenoic acids.

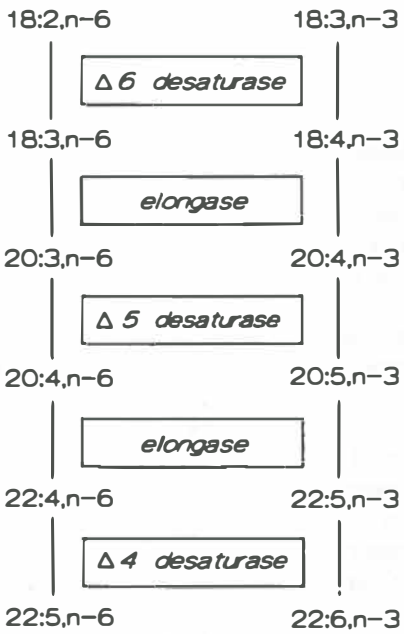


Arachidonic acid (20:4,n-6) Eicosapentaenoic acid (20:5,n-3)

chylomicrons as a precursor for the biosynthesis of 20:4,n-6 by microsomal desaturation and chain elongation. In addition, the liver modifies and secretes (via very low density lipoproteins) fatty acids that were taken up from the blood stream or *de novo* synthesized from non fatty acid precursors. Tissues may use these fatty acids as structural building blocks after further modification, if necessary. Cells may also receive 18:2,n-6 from adipose tissue, which in the fasting state releases non-esterified fatty acids.

The enzyme "delta-6 desaturase" converts 18:2,n-6 into gamma-linolenic acid (18:3,n-6), which constitutes the rate limiting step in the conversion of 18:2,n-6 into 20:4,n-6 (Figure 2). In the following reaction 18:3,n-6 is converted into di-homo-gamma-linolenic acid (20:3,n-6) by a chain elongase. The "delta-5 desaturase" subsequently introduces another double bond into 20:3,n-6 to produce 20:4,n-6. Analogously, 18:3,n-3 can be converted into 20:5,n-3 and finally into 22:6,n-3. The possibility to retroconvert 22:6,n-3 into 20:5,n-3 has led to the notion that the former may serve as a reservoir of the latter[42].

Figure 2 Fatty acid desaturation and chain elongation of linoleic and alpha-linolenic acids.

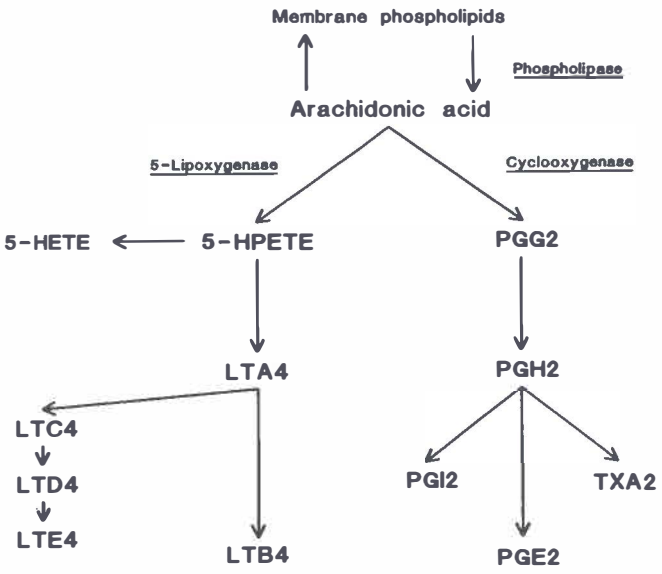


Eicosanoids

Eicosanoids are oxygenation products of metabolism of PUFA with 20 carbon atoms, of which 20:4,n-6 is the most important. Membrane phospholipids are considered to be the predominant pool of precursor fatty acids for eicosanoid synthesis. Eicosanoids play

essential roles in human haemostasis, immune response, and tissue perfusion[43]. The formation of the major eicosanoids occurs through two different enzymatic pathways. The cyclooxygenase pathway leads to the production of prostaglandins, thromboxanes and prostacyclins, whereas the lipoxygenase pathway, leads to the production of leukotrienes. (Figure 3)

Figure 3 Simplified scheme of the arachidonic acid metabolism

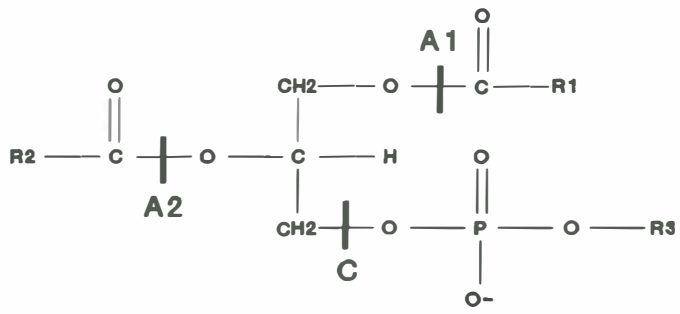


Release of arachidonic acid

The rate limiting step in the formation of eicosanoids is the cleavage of 20:4,n-6 from its mostly sn-2 position in membrane phospholipids by a phospholipase (Figure 4). Activation of membrane receptors may enhance phospholipase activity. The receptor signal is transmitted to the phospholipases through G-proteins. Phospholipases A2 (PL-A2) and C (PL-C) are assumed to be most commonly involved in this process. Other phospholipases like PL-A1 can initiate the release 20:4,n-6 from membrane phospholipids as well. PL-A1 activity leads to the production of a 2-acyl lyso-phospholipid and requires the subsequent action of a lysophospholipase to release 20:4,n-6. During PL-A2 activity, 20:4,n-6 is directly released with a 1-acyl lyso-phospholipid as co-product. The latter is lytic to cell membranes and is either reacylated or degraded to water soluble products[44]. The activity of PL-A2 is Ca^{2+} dependent[45]. If PL-A2 acts upon alkenyl ether containing phospholipids (plasmalogens), then lyso-platelet activating factor (lyso-PAF) is produced, which, via acylation, is converted into PAF. PL-C activity is primarily exerted on phosphatidyl-inositol-bis-phosphanate (PIP2). The PL-C action is independent of the presence of Ca^{2+} and leads finally to the formation of the triphosphorylated form of inositol, (IP3), and diacylglycerol (DAG). Subsequently 20:4,n-6 can be cleaved from DAG by a DAG-lipase. IP3 stimulates the

release of Ca^{2+} from intracellular stores, whereas DAG activates protein kinase C which in turn modulates the extent of 20:4,n-6 mobilized[44].

Figure 4 Generalized structure of a phospholipid and the bonds attacked by different phospholipases.



R1 and R2 represent the aliphatic chains of fatty acids and R3 represents some polar head group like choline, serine, ethanolamine and inositol. Fatty acids are removed from the sn-1 position by phospholipase A1 and from the sn-2 position by phospholipase A2. Phospholipase C removes the whole head of the group leaving diacylglycerol.

The cyclooxygenase pathway

The enzyme cyclooxygenase catalyses the oxygenation of free 20:4,n-6 into a 15-hydroperoxy endoperoxide (prostaglandin G2; PGG2). Subsequently a peroxidase catalyses the conversion of PGG2 to a transient hydroxy-endoperoxide (prostaglandin H2; PGH2). Dependent upon the cell type being stimulated, the intermediates can either be transformed into prostaglandins, thromboxanes or prostacyclins. After stimulation of platelets, the formation of thromboxanes (TX) is set on. The characteristic properties of TXA2 contribute to aggregation by promoting vasoconstriction and activation of platelets to release vasoactive substances. Formation of prostacyclin I2 (PGI2) usually takes place in endothelial cells. Because of its vasodilating and anti-aggregating properties, PGI2 is the counterbalance of TXA2. Prostaglandin E2 (PGE2) is the main product of the cyclooxygenase pathway in human leukocytes. It modulates the immune response and possesses anti-inflammatory activities as well (Table 2). It has been shown that cultured fibroblasts dispersed from the rheumatoid synovium produce large amounts of PGE2[55]. Interleukins are able to induce PGE2 synthesis. Binding of IL-1 to the membrane receptor leads to an increase in PL-A2 activity and synthesis of the cyclooxygenase enzyme[56]. In turn, PGE2 inhibits the macrophage derived production of IL-1 and TNF. Preexposure of macrophages to PGE2 can desensitize these cells to the effects of subsequent PGE2 on the lipopolysacharide-induced production of TNF[48]. Evidently, the mediation and regulation of cytokine synthesis remains unclear and more extensive studies are needed to address this issue.

The lipoxygenase pathway

By inserting molecular oxygen at C-5 of 20:4,n-6, the enzyme '5-lipoxygenase' oxidizes 20:4,n-6 to 5S-hydroperoxy-6,8-trans-11,14-cis-eicosatetraenoic acid (5-HPETE) which in turn is converted into 5,6 trans-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid (LTA4). The enzyme 5-lipoxygenase is the key enzyme in leukotriene biosynthesis. In

resting cells it is associated with the cytosol. Upon cell stimulation the enzyme is translocated and becomes associated with the cell membrane. Calcium and ATP are required for maximal activity of 5-lipoxygenase while additionally some cytosolic and membrane bound proteins (e.g. 5-lipoxygenase activating protein (FLAP)) facilitate enzyme activity[57,58]. 5-Lipoxygenase expression appears to be restricted to cells of myeloid linage. In contrast, LTA4 hydrolase activity, has been detected in leukocytes, erythrocytes, lymphocytes and fibroblasts and endothelial cells as well[59]. The LTA4 hydrolase converts the unstable intermediate LTA4 to 5S,12R-dihydroxy-6,14-cis8,10-trans-eicosatetraenoic acid (LTB4). By transcellular transport, monocyte derived LTA4 was shown to be converted, by LTA4 hydrolase from B-cells, into LTB4. Thus, LTB4 can also be generated by cells which do not contain the 5-lipoxygenase enzyme. Finally, LTB4 is inactivated by sequential oxidation of its terminal carbon which leads to the formation of 20-COOH-LTB4[60].

It is assumed nowadays that leukotrienes are involved in a variety of acute and chronic inflammatory responses *in vivo*[60,61] (Table 2). They exert a broad range of biological actions, including effects on leucocyte migration, aggregation, adherence to vascular endothelium *in vitro*[62] and *in vivo*[63], enzyme secretion and smooth muscle cell contraction and vascular permeability[64]. In addition, the leukotrienes have been implicated in the regulation and mediation of cytokine synthesis[73].

Table 2 Some biological properties of prostaglandin E2 and leukotriene B4.

Prostaglandin E2	
	Potentiates:
	- Blood flow at the site of inflammation
	- Vascular permeability
	- Development of fever
	- Pain (in synergy with bradykinin)
	Inhibits:
	- Interleukin-1 production (macrophages) [46,47]
	- Tumor necrosis factor production (macrophages) [48]
	- Interleukin-2 production (T-cells) [49]
	- Gamma-interferon production (T-cells) [50]
	- Interleukin-2 receptor expression (T-cells) [51]
	- MHC class II expression (monocytes) [52]
	- T-suppressor cell activity [53]
	- B-cell proliferation and activation [53,54]
	- Natural killer cell activity [53]
Leukotriene B4	
	Potentiates:
	- Leucocyte migration and aggregation [65]
	- Leucocyte endothelial adherence [66]
	- Oxidative metabolism (neutrophils) [67]
	- C3b complement receptor expression (neutrophils) [68]
	- T-suppressor cell proliferation and activation [69]
	- Interleukin-1 production (macrophages) [70]
	- Interleukin-2 production (T-cells) [70]
	- Gamma-interferon production (T-cells) [71,72]

Influences of some pharmacologic agents on eicosanoid synthesis

NSAID have antiinflammatory, analgesic and antipyretic properties. The conversion of 20:4,n-6 to PGG2 via the cyclooxygenase pathway is inhibited by most NSAID. This inhibition may on the one hand be therapeutic but on the other hand accounts for many of the side effects of NSAID. For instance, prostaglandins have a protective effect on gastric mucosa, and are involved in the regulation of renal blood flow and sodium and water balance. Consequently, blockage of prostaglandin production may lead to ulcers in the upper gastrointestinal tract and impairment of renal function. Furthermore, inhibition of the cyclooxygenase pathway may cause shifting of 20:4,n-6 to the lipoxygenase pathway, resulting in increased production of leukotrienes and intermediates, that is suggested to induce e.g. aspirin intolerant asthma[74]. In general, NSAID do not affect the production of leukotrienes. Exceptions are timegadine and tolfenamic acid[75] that may affect the lipoxygenase pathway. Controversy persists about whether inhibition of prostaglandin synthesis is the specific underlying mechanism of NSAID action[21,76]. This is illustrated by the fact that nonacetylated salicylates are poor inhibitors of prostaglandin synthesis, but are yet effective in suppressing joint symptoms in RA patients. Additional support is provided by the finding that small doses of NSAID are needed to inhibit prostaglandin synthesis, while much higher doses are required to achieve beneficial effects in RA. Some studies have demonstrated variable depression of neutrophil aggregation, adhesiveness, chemotaxis and degranulation[77], and inhibition of superoxide anion generation contributing to the NSAID antiinflammatory effects.

Drugs that primarily affect the action or the production of leukotrienes are under development. Examples of such drugs currently tested in human experimental clinical trials are MK-571, a LTD4 receptor antagonist, and MK-886. The latter inhibits the translocation of 5-lipoxygenase to the membrane where the enzyme would be activated[78]. It seems that lipoxygenase inhibitors may offer a promise as a therapeutic agent in inflammation, but much work has to be done before their role can be defined.

Glucocorticoids are principal therapeutic agents in the management of many inflammatory and autoimmune diseases. Lipocortins are a mixture of proteins with phospholipase and calcium binding activities. After administration of therapeutic dosages of glucocorticoids, lipocortins are translocated to the cell surface and block eicosanoid synthesis. Although the glucocorticoid induced effects seem at least in part to be mediated by lipocortins, the mechanism is still controversial[79].

Influence of dietary n-3 PUFA on the fatty acid composition of plasma- and cell lipids

Plasma lipid fatty acid composition:

Certain vegetable oils, like soybean and canola oil, provide a significant source of n-3 PUFA[80]. The predominant n-3 PUFA found in these oils is 18:3,n-3. Large amounts of n-3 PUFA with 20 carbon atoms or more are obtained by eating fatty fish. Especially mackerel, salmon, sardines, anchovy and herring, are rich sources of 20:5,n-3 and 22:6,n-3. In the last decade refined fish oils have become available. As these oils offer the opportunity to preferentially provide long chain n-3 PUFA one avoids excessive intake of other fatty acids, vitamins and toxic agents.

The fish oil fatty acids can either be provided in their natural triglyceride form or in their semisynthetic methyl- and ethyl ester forms. The methyl and ethyl ester forms allow for higher relative amounts of n-3 PUFA and contain lower amounts of cholesterol and saturated fatty acids than fish oil in its natural triglyceride form. In some trials an incomplete absorption of n-3 PUFA has been demonstrated when the fish oil was provided in ethyl ester form[81-83]. In contrast, others have demonstrated that ingestion of fish oil triglyceride and ethyl ester preparations containing equal amounts of n-3 PUFA enriched plasma lipids with 20:5,n-3 to the same extent[84].

Once the n-3 PUFA are absorbed they are readily incorporated into the plasma lipid fractions. A fish oil enriched diet leads to a substantial increase in the relative amounts of 20:5,n-3 and 22:6,n-3 in each of the plasma lipid fractions (phospholipids, triglycerides, cholesterol esters). The increase is dose dependent and occurs predominantly at the expense of n-6 PUFA, such as 18:2,n-6 and 20:4,n-6. Recently, Bilo et al[85] demonstrated that the relative amount of 20:5,n-3 in plasma phospholipids (3.65% of total fatty acids) hardly increases after doubling the dose of fish oil fatty acid ethyl esters from 3 to 6 g per day. However, much higher levels of 20:5,n-3 in the plasma phospholipids fraction (up to 30%) were found by others[86]. The discrepancy between the studies is difficult to ascertain, but differences in the chemical form in which the fish oil fatty acids were provided, background diet, or both, may explain this phenomenon.

Cell membrane phospholipid fatty acid composition

The PUFA composition of the phospholipid fraction of various cells responds to changes in the dietary fatty acid content (Table 3). Changes are dependent upon the

Table 3 Relative amounts of eicosapentaenoic and arachidonic acids in the total phospholipid fraction of various cell types. Data were obtained from several studies assessing the effects of dietary fish oil supplementation.

	20:5,n-3		20:4,n-6		Dosage	Duration
	Before	After	Before	After	n-3 PUFA	of therapy
Platelets						
Goodnight et al[87]	0.1	6.1	27.6	20.2	10.0 g EPA + DHA	4 weeks
Von Schacky et al[88]	0.05	4.3	26.1	20.5	3.8 g EPA + 5.5 g DHA	12 weeks#
Dyerberg et al[89] (Greenland Eskimo)	.	8.0	.	8.5	10.0 g EPA + DHA	Life time
Erythrocytes						
Knapp et al[90] •	0.4	6.2	6.5	5.0	10.0 g EPA	4 weeks
Knapp et al[90] ••	0.4	4.5	24.3	19.5	10.0 g EPA	4 weeks
Cartwright et al[91]	1.1	2.9	14.1	14.1	1.9 g EPA + 1.2 g DHA	6 weeks
Monocytes						
Lee et al[92]	0.09	1.5	6.7	9.4	3.2 g EPA + 2.2 g DHA	6 weeks
Endres et al[93]	0.7	3.8	13.8	8.6	2.8 g EPA + 1.9 g DHA	6 weeks
Neutrophils						
Lee et al[92]	0.09	0.7	4.1	5.9	3.2 g EPA + 2.2 g DHA	6 weeks
Tempel vd et al[94]	0.2	0.8	5.2	4.3	2.0 g EPA + 1.3 g DHA	12 weeks
Sperling et al[95]	0.2	3.8	15.1	10.2	3.6 g EPA + 2.4 g DHA	6 weeks
Arm et el[96]	0.2	2.6	14.6	13.3	3.2 g EPA + 2.2 g DHA	10 weeks

The amount of 20:5,n-3 and 22:6,n-3 (40 ml Cod liver oil/day) stepwise increased from 0 via 10 and 20 ml cod liver oil/ day each 4 weeks. • Phosphatidylcholines, •• Phosphatidylethanolamines

half life of both the cells and their lipids. Appreciable amounts of 20:5,n-3 and 22:6,n-3 appear in plasma phospholipids already four hours after dietary fish oil ingestion, but in platelet phospholipids it was not until day 6[97]. Such a finding illustrates the fact that n-3 PUFA accumulation in membrane phospholipids is not accomplished by exchange with surrounding plasma species only, but is principally embedded during cell maturation. It can, however, not be excluded that some tuning of the fatty acid composition of cell membranes occurs by phospholipid exchange with other organelles and plasma lipoproteins[98,99]. The range over which the PUFA composition of membrane phospholipids varies is, generally, restricted to the substitution of one PUFA for another on the sn-2 positions[100]. The distribution of the n-3 PUFA over the different phospholipid subfractions comprising phosphatidyl-choline, -ethanolamine, -inositol and -serine may, however not be proportional[90]. Dietary n-3 PUFA e.g. interact with the n-6 PUFA modification. By competition for the delta-5 and delta-6 desaturase enzymes, n-3 PUFA inhibit the conversion of 18:2,n-6 to 20:4,n-6[101,102]. Surprisingly the influence of n-3 PUFA on cellular amounts of 20:4,n-6 is rather unpredictable. Some studies mention a decrease[95], whereas others do not[92] (Table 3).

Influence of dietary n-3 PUFA on the production of eicosanoids

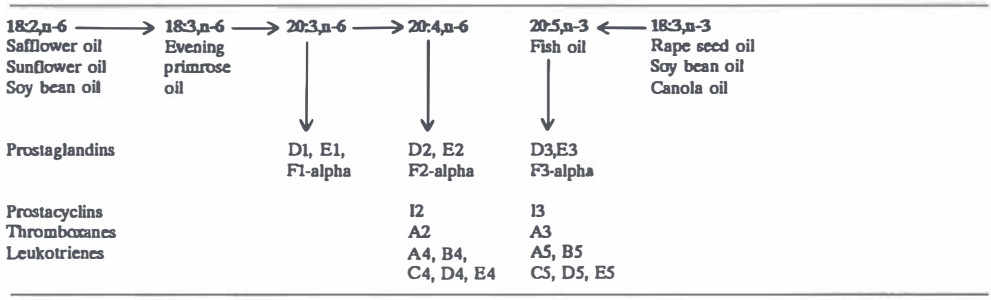
Dietary n-3 PUFA supplementation can suppress the 20:4,n-6 derived eicosanoid synthesis in several types of cells. In this respect 20:5,n-3 is considered to be the most active substance of the n-3 PUFA. By partial ß-oxidation, 22:6,n-3 serves a reservoir for the formation of 20:5,n-3[42]. The 20:5,n-3 is presumed to affect the production of different types of 20:4,n-6 derived eicosanoids in several ways[103]: *first*, by displacing 20:4,n-6 from the metabolically active phospholipid pools, 20:5,n-3 reduces precursor availability; *second*, the release of 20:4,n-6 from these pools may be diminished[104] and *third*, released 20:5,n-3 competes with 20:4,n-6 for cyclooxygenase and lipoxygenase enzymes but it is poorly metabolized to its endproducts[105].

Prostaglandins, prostacyclins and thromboxanes

Dietary fish oil supplementation causes a substantial inhibition of platelet TXA2 production, while the production of PGI2 by endothelial cells is slightly decreased or remains stable[98,106]. Concomitantly, a moderate production of TXA3 and PGI3 becomes detectable (Figure 5). TXA3 is a weak agonist of TXA2 in inducing vasoconstricting and platelet aggregation. In comparison with PGI2, PGI3 exerts similar vasodilating and anti-aggregating effects[107]. In humans, (patients with essential hypertension and healthy volunteers) high dose fish oil (9 g 20:5,n-3 + 6 g 22:6,n-3) administered over 4 weeks, led to a 50% fall in the urinary excretion of TXA2 metabolites, while low amounts of TXA3 metabolites became detectable. The excretion of PGI2 metabolites moderately decreased in patients only, whereas PGI3 metabolite excretion increased in both patients and controls[108]. These data indicate a shift of the PGI2/TXA2 balance towards a more antiaggregatory and vasodilatory state during n-3 PUFA supplementation. Such a mechanism may account for prolonged bleeding times and contribute to the low mortality from coronary heart disease during consumption of n-3 PUFA. Although no consistent suppression of the urine excretion of PGE2 metabolites was noted, alterations in cellular capacity to produce prostaglandins during fish oil supplementation are more clear. Endres et al[93] demonstrated a 51% decrease of

PGE2 production by mononuclear cells isolated from healthy volunteers who were supplemented with 2.7 g 20:5,n-3 + 1.8 g 22:6,n-3/day for six weeks. In contrast, in patients with persistent asthma, 4 g of 20:5,n-3/day for 8 weeks did not alter the total mononuclear prostaglandin production of the E series. But a moderate suppression of PGE2 production became detectable when PGE2 was resolved from PGE3 by radio immunoassay[109]. An extensive inhibition of the PGE2 production (70%) was observed in synovial explant cultures of fish oil-fed rats with a collagen-induced arthritis. The n-3 PUFA were, however, the only source of dietary fat administered to the animals[110].

Figure 5 Transformation of 20:3,n-6, 20:4,n-6 and 20:5,n-3 into various cyclooxygenase and lipoxygenase products.



Leukotrienes

5-Lipoxygenase can convert 20:5,n-3 into LTB5, LTC5, LTD5 and LTE5 (Figure 5). The 22:6,n-3 can be metabolized into 7- and 4-hydroperoxy 22:6,n-3 and their reduction products, only[111-113]. Formation of 20:5,n-3 derived leukotrienes is associated with an inhibition of the enzymatic conversion of 20:4,n-6 into leukotrienes[111]. This has been explained by both diminished release of 20:4,n-6 from its esterified pools and diminished function of 5-lipoxygenase. The latter was suggested because of the reduced generation of the sequential 20:4,n-6 derived reaction products, 5-HETE, LTA4 and LTB4[92]. Sperling et al[95], however, alleged a selective inhibition of the LTA4 hydrolase activity as the mechanism of action of 20:5,n-3 on leukotriene production. They noted that the generation of 5-HETE and 5-HEPE (precursors of LTB4 and LTB5 respectively) was unchanged, whereas the sum of LTB4 and LTB5 generation was suppressed during fish oil supplementation[95] (Table 4).

Table 4 Generation of 5-lipoxygenase pathway products (ng/10^6 cells) by calcium ionophore stimulated neutrophils from patients with rheumatoid arthritis before and after 6 weeks of dietary fish oil (3.6 g 20:5,n-3 + 2.4 g 22:6,n-3) supplementation.

	Before	After
5-HETE	26.4	21.8
LTB4	26.0	17.4
5-HEPE	.	6.9
LTB5	.	2.6
5-HETE + 5-HEPE	26.4	28.7
LTB4 + LTB5	26.0	19.9

Data were obtained from reference 195.

LTB5 is 10-30 times less potent than LTB4 in eliciting a response from neutrophil chemotaxis[113,114] and enhancing complement receptors *in vitro*[113]. This has been attributed to the rigidity of the C17-C18 bond in LTB5[113]. In contrast to LTB5, LTC5 is approximately equipotent to LTC4[115]. The optimum amount of n-3 PUFA required to alter leukotriene synthesis is, as yet, unknown. Administration of n-3 PUFA (10% by weight) to male Wistar rats reduced the relative amounts of 20:4,n-6 in leukocyte lipids with 35%[116]. This was accompanied by a 50% decrease of the LTB4- and a concomitant increase of the LTB5 production. In RA patients daily ingestion of 3.6 g 20:5,n-3 + 2.4 g 22:6,n-3 during a 6 weeks period led to a 33% suppression of the LTB4 formation, while small quantities of LTB5 became detectable[95]. Lower dosages of fish oil were found effective in altering the leukotriene metabolite pattern as well[94].

Dietary fish oil supplementation in rheumatoid arthritis

The rationale of fish oil supplementation in rheumatoid arthritis is based on the assumption that prostaglandins and leukotrienes are actively involved in the process of joint inflammation. For instance, local production of LTB4 in the rheumatic joint may cause attraction of several kinds of cells that contribute to the inflammatory destruction of cartilage and increased bone loss[117,118]. In addition, neutrophils from RA patients may have an increased capacity to convert endogenous 20:4,n-6 into LTB4[119]. As dietary n-3 PUFA modulate the production of eicosanoids, this may have consequences for a chronic inflammatory disorder like RA. In fish oil fed mice, a reduction of the severity of 'type II collagen' induced arthritis was observed[120]. In another study it was shown that five weeks after arthritis induction in susceptible mice, acute phase protein production was lower in fish oil fed mice when compared with corn oil fed mice[121]. The effects of fish oil supplementation in animal models may, however, be dependent on gender and animal species, since not all studies have shown unanimous beneficial effects[122]. In 1985 Kremer and associates[123] were the first to report that a diet high in PUFA and low in SAFA supplemented with 1.8 g 20:5,n-3 daily resulted in a decrease of duration of early morning stiffness and number of tender joints in RA patients. Comparisons were made with a typical American diet supplemented with placebo. The 20:5,n-3 supplemented diet had beneficial effects on joint symptoms. However, some of the significant differences in clinical parameters were due to a combination of improvement in the experimental group and deterioration in the controls.

Recently, Pike[124] argued: *'When considering the limited spectrum of the current therapeutic armamentarium of nonspecific immune-suppressive agents available to rheumatologists for the treatment of inflammatory disorders, it would appear that more extensive, double blind trials evaluating dietary modification with plant and/or marine lipids are warranted'.*

3. AIM OF THIS THESIS

The aim of this thesis is to answer the following questions:

1) Does dietary fish oil supplementation affect the usual clinical and laboratory disease variables in RA patients.
2) What kind of changes does fish oil supplementation induce in leukotriene production by isolated neutrophils.
3) Is there a relationship between alterations in the production of leukotrienes and changes in disease variables.
4) To what extent is the accumulation of fish oil fatty acids in plasma and cell lipids influenced by the additional dietary intake of 18:2,n-6 and the chemical form in which the fish oil fatty acids are provided.

Chapter 1 includes an introduction to rheumatoid arthritis, dietary fish oil and eicosanoid synthesis. *Chapter 2* describes the effects of dietary fish oil supplementation on clinical and laboratory disease variables and in vitro leukotriene production in RA patients. *Chapter 2A* describes a letter to the editor concerning the influence of fish oil supplementation on IL-1 production. *Chapter 3* Deals with the influence of dietary intake of fish oil fatty acids on vitamin E status parameters. A study on the effects of fish oil treatment on the NSAID demand in RA is presented in *Chapter 4*. In *Chapter 5* we compared the efficacy of 'high' dose fish oil fatty acid ethyl ester and 'low' dose fish oil triglyceride preparations for altering fatty acid compositions of plasma cholesterol esters and neutrophil phospholipids, neutrophil leukotriene production and disease activity. The effect of dietary energy percentage of fat and its PUFA/SAFA ratio on incorporation of n-3 PUFA and leukotriene production during dietary fish oil supplementation was studied in healthy volunteers (*Chapter 6*). Finally, in *Chapter 7* the present state-of-the-art on fish oil supplementation in RA is reviewed.

REFERENCES

1. Harris ED. The clinical features of rheumatoid arthritis. In Kelly Wn, Harris ED, Ruddy S, Sledge CD eds. Textbook of rheumatology. Philadelphia WB Saunders, 1989:943-981.
2. Ropes MW, Bennet GA, Cobb S, Jacox R, Jessar RA. Proposed diagnostic criteria for rheumatoid arthritis. Bull Rheum Dis 1956;7:121-124.
3. Ropes MW, Bennet GA, Cobb S, Jacox R, Jessar RA. 1958 revison of diagnostic criteria for rheumatoid arthritis. Bull Rheum Dis 1958;9:175-176.
4. Arnett FC, Edworthy SM, Bloch DA et al. The american rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-324.
5. Chen TP, Fong T, Carson DA. Rheumatoid factor, Rheum Dis Clin North Am 1987;13:545-568.
6. Ritchie DM, Boyle JA, Mc Innes JM, Jasani MK, Dalakos T, Grieveson P, Buchanan W. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. Quart J Med 1968;147:393-406.
7. Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum 1980;23:137-145.
8. Meenan RF, Gertman PM, Mason JM. Measuring health status in arthritis. Arthritis Rheum 1980;2:146-152.

9. Taal E, Jacobs JWG, Seydel ER, Wiegman O, Rasker JJ. Evaluation of the Dutch Arthritis Impact Measurement Scale (Dutch-AIMS) in patients with rheumatoid arthritis. Br J Rheumatol 1989;28:487-491.
10. Harris ED. Rheumatoid arthritis, Pathophysiology and implications for therapy. N Engl J Med 1990;322:1277-1289.
11. Zvaifler NJ. An introduction to rheumatoid arthritis. Arthritis Care Research 1989 s17-22.
12. Winchester R, Gregersen PK. Molecular basis of susceptibility to rheumatoid arthritis. Springer Sem Immunopathol 1988;10:119-139.
13. Alspaugh MA, Henle G, Lenette ET, Henle W. Elevated levels of antibodies to Epstein-Barr virus antigens in sera and synovial fluids of patients with rheumatoid arthritis. J Clin Invest 1981;67:1134-1140.
14. Silverman SL, Schumacher HR. Antibodies to Epstein-Barr viral antigens in early rheumatoid arthritis. Arthritis Rheum 1981;24:1465-1468.
15. Korst van der JK. Een naald in de hooiberg; de rol van voedingsfactoren in de pathogenese van reumatoide artritis. Ned Tijdschrift Geneesk 1986;130:1428-1430.
16. Arend WP, Dayer JM. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. Arthritis Rheum 1990;33:305-315.
17. Heide van der DMFM, Riel van PLCM, Rijswijk vna MH, Putte van de LBA. Influence of prognostic features on the final outcome in rheumatoid arthritis: A review of the literature. Sem Arthritis Rheum 1988;4:284-292.
18. Rasker JJ, Cosh JA. Cause and age at death in a prospective study of 100 patients with rheumatoid arthritis. Ann Rheum Dis 1981;40:115-120.
19. Vandenbroucke JP, Hazevoet HM, Cats A. Survival and cause of death in rheumatoid arthritis. J Rheumatol 1984;11:158-161.
20. Mitchel DM, Spitz PW, Young DY, Bloch DA, McShane DJ, Fries JF. Survival, prognosis, and causes of death in rheumatoid arthritis. Arthritis Rheum 1986;6:706-714.
21. Abramson SB, Weissmann G. The mechanisms of action of non steroidal antiinflammatory drugs. Arthritis Rheum 1989;1:1-9.
22. Rijthoven van AWAM, Dijkmans BAC, Goei The HS, et al. Cyclosporine treatment for rheumatoid arthritis: A placebo-controlled double-blind multicentre study. Ann Rheum Dis 1986;45:726-731.
23. Breedveld FC, Dijkmans BAC, Mattie H. Minocycline treatment for rheumatoid arthritis: an open dose finding study. J Rheumatol 1990;17:43-46.
24. Homan van der Heide JJ, Bilo HJG, Tegzess AM, Donker AJM. The effects of dietary supplementation with fish oil on renal function in ciclosporin-treated renal transplant recipients. Transplantation 1990;3:523-527.
25. Kowsari B, Finnie SK, Carter RL, et al. Assessment of the diet of patients with rheumatoid arthritis and osteoarthritis. J Am Diet Assoc 1983;82:657-659.
26. Collins R, Dunn TL, Walthaw J, Harrell P, Alarcon GS. Malnutrition in rheumatoid arthritis. Clin Rheumatol 1987;6:391-398.
27. Committee on Dietary Alowances, Food and Nutrition Board, Recommended Dietary Allowances. National Academy of Sciences. 9th ed. Washington, D.C. 1980.
28. Bjarnason I, Williams P, So A et al. Intestinal permeability and inflammation in rheumatoid arthritis: effects of non-steroidal anti-inflammatory drugs. Lancet 1984;I:1171-1173.
29. Grindulis KA, McConkey B. Does sulphasalazine cause folate deficiency in rheumatoid arthritis? Scand J Rheumatol 1985;14:265-270.
30. Panush RS. Food induced ('allergic') arthritis: clinical and serologic studies. J Rheumatol 1990;17:291-294.
31. Panush RS, Carter RL, Katz P, Kowasri B, Longley S, Finnie S. Diet therapy for rheumatoid arthritis. Arthritis Rheum 1983;26:462-471.
32. Hafstrom I, Ringertz B, Gyllenhammar H, Palm blad J, Harms-Ringdal M. Effects of fasting on disease activity, neutrophil function, fatty acid composition, and leukotriene biosynthesis in patients with rheumatoid arthritis. Arthritis Rheum 1988;5:585-592.

33. Uden AM, Trang L, Venizelos N, Palmblad J. Neutrophil functions and clinical performance after total fasting in patients with rheumatoid arthritis. Ann Rheum Dis 1983;42:45-51.
34. Belch JJF, O'Dowd A, Ansell D, Sturrock RD. Leukotriene B4 production by peripheral blood neutrophils in rheumatoid arthritis. Scand J Rheumatol 1989;18:213-219.
35. Bang HO, Dyerberg J. Plasma lipids and lipoproteins in greenlandic west coast eskimos. Acta Med Scand 1972;192:85-94.
36. Bang HO, Dyerberg J, Hjorne N. The composition of food consumed by greenland eskimos. Acta Med Scand 1976;200:69-73.
37. Kromhout D, Bosschieter EB, Lezenne Coulander de C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Eng J Med 1985;312:1205-09.
38. Kremer JM, Jubiz W, Michalek AV, et al. Fish oil fatty acid supplementaion in active rheumatoid arthritis. Ann Intern Med 1987;106:497-503.
39. Crawford MA, Hassam AG, Williams G. Essential fatty acids and fetal brain growth. Lancet 1976;28:452-453.
40. Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effect of prenatal and postnatal omega-3 fatty acid deficiency on retina and brain in rhesus monkeys. Proc Natl Acad Sci USA 1986;83:4021-4025.
41. Holman RT. Essential fatty acid deficiency in humans. In Rechgli Jr, M, Ed. CRC handbook series in nutrition and food. Section E: Nutritional disorders. Vol III Effect of nutrient deficiencies in man. CRC Press, Inc, West Palm Beach, Florida, USA, 1978;335-368.
42. Hagve T, Christophersen BO. Evidence for peroxismal retroconversion of adrenic acid (22:4 (n-6)) and docosahexaenoic acids (22:6 (n-3)) in isolated liver cells. Biochim Biophys Acta 1986:165-173.
43. Cannon PJ. Eicosanoids and the blood vessel wall. Circulation 1984;70:523-528.
44. Dennis EA. Phospholipase A2 mechanism: inhibition and role in arachidonic acid release. Drug Develop Res 1987;10:205-220.
45. Balsinde J, Fernandez B, Diez E. Regulation of arachidonic acid release in mouse peritoneal macrophages. The role of extracellular calcium and protein kinase C. J Immunol 1990;11:4298-4304.
46. Knudsen PJ, Dinarello CA, Strom TB. Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin-1 activity by increasing intracellular cyclic adenosine monophosphate. J Immunol 1986;137:3189-3194.
47. Kunkel SL, Chensue SW, Phan SH. Prostaglandins as endogenous mediators of interleukin 1 production. J Immunol 1986;136:186-192.
48. Farrar WL, Humes JL. The role of arachidonic acid metabolism in the activities of interleukin 1 and 2. J Immunol 1985;135:1153-1159.
49. Chouaib S, Fradelizi D. The mechanism of inhibition of human IL-2 production. J Immunol 1982;129:2463-2468.
50. Hasler J, Bluestein HG, Zvaifler NJ, Epstein LB. I Analysis of the defects responsible for the impaired regulation of EBV-induced B-cell proliferation by rheumatoid arthritis lymfocytes. Diminished gamma-interferon production in reponse to autologous stimulation. J Exp Med 1983;157:173-188
51. Chouaib S. Robb RJ, Welte K, Dupont B. Analysis of prostaglandin E2 effect on T-lymphocyte activation. Abrogation of prostaglandin E2 inhibitory effect by the tumor promotor 12.0 tetradecanoyl phorbol-13 acetate J Clin Invest 1987;80:333-340.
52. Trip CS, Wyche A, Unnanus ER, Needleman P. The functional significance of the regulation of macrophage Ia expression by endogenous arachidonate metabolites in vitro. J Immunol 1986;137:3915-3920.
53. Staite ND, Panayi GS. Regulation of human immunoglobulins production in vitro by prostaglandin E2. Clin Exp Immunol 1982;49:115-122.
54. Alvarellos A. Lipsky PE, Jasin HE. Prostaglandin E2 modulation of rheumatoid factor synthesis. Arthritis Rheum 1988;31:1473-1480.
55. Dayer JM, Krane SM, Russell GG, Robinson DR. Production of collagenase and prostaglandins by isolated adherent synovial cells. Proc Natl Acad Sci 1976;73:945-949.

56. Burch RM, Connor JR, Axelrod J. Interleukin 1 amplifies receptor-mediated activation of phospholipase A2 in 3t3 fibroblasts. Proc Natl Acad Sci USA 1988;85:6306-6309.
57. Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reaction and inflammation. Science 1983;220:568-575.
58. Lewis RA, Austen F, Soberman RJ. Leukotrienes and other products of the 5-lipoxygeanse pathway. Biochemistry and relation to pathobiology in human diseases. N Engl J Med 1990;10:645-654.
59. Samuelsson B, Claesson HE. Leukotriene B4: Biosynthesis and role in lymphocytes. In Samuelsson B, Dahlen SE, Fritsch J, Hedqvist P eds. Advances in prostaglandin, thromboxane and leukotriene research. Trends in eicosanoid biology. New York Raven Press 1990:1-13.
60. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis and biological effects. Science 1987;237:1171-1175.
61. Parker CW. 5-Lipoxygenase, leukotrienes and regulation of inflammatory responses. Drug Develop Res 1987;10:277-293.
62. Hoover RL, Karnovsky MJ, Austen KF, Corey EJ, Lewis RA. Leukotriene B4 action on endothelium mediates augmented neutrophil/endothelial adhesion. Proc Natl Acad Sci 1984;81: 2191-2193.
63. Bray MA, Ford-Hutchinson AW, Smith MJH. Leukotriene B4: an inflammatory mediator in vivo. Prostaglandins 1981;22:213-222.
64. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJH. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 1980;286:264-265.
65. Payan DG, Goetzl EJ. Specific suppression of human T lymphocyte function by leukotriene B4. J Immunol 1983;131:551-553.
66. Renkonen R, Mattila P, Pleszczynski D, Hayry P. Leukotriene B4 increase the lymphocyte binding to endothelial cells. Fed Eur Biochim Soc 1988;235:67-70.
67. Palmblad J, Gyllenhammar H, Lindgren JA, Malmsten CL. Effects of leukotrienes and F-Met-Leu-Phe on oxidative metabolism of neutrophils and eosinophils. J Immunol 1984;132:3041-3046.
68. Nagy L, Lee TH, Pickett WC, Kay AB. Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. Clin Exp Immunol 1982;47:541-547.
69. Gualde N, Atluru D, Goodwin JS. Effect of lipoxygenase metabolites of arachidonic acid on proliferation of human T-cell subsets. J Immunol 1985;134:1125-1129.
70. Rola-plezczynski M, Lemaire I. Leukotrienes augment interleukin 1 production by human monocytes. J Immunol 1985;135:3958-3961.
71. Pleszcynski MR, Bouvrette L, Gingras D, Girard M. Identification of interferon as the lymphokine that mediated leukotriene B4 induced immunoregulation. J Immunol 1987;139:513-517.
72. Johnson HM, Torres BA. Leukotrienes: positive signals for regulation of gamma-interferon production. J Immunol 1984;132:413-416.
73. Hopkins SJ. Cytokines and eicosanoids. Ann Rheum Dis 1990;49:207-211.
74. Slepian IK, Mathews KP, McLean JA: Aspirin sensitive asthma. Chest 1985;87:386-391.
75. Moilanen E, Alanko J, Seppl, Vapaatalo H. Effects of antirheumatic drugs on leukotriene B4 and prostanoid synthesis in human polymorphonuclear leukocytes in vitro. Agents Actions 1988;24:387-394.
76. Altman RD. Neutrophil activation: An alternative to prostaglandin inhibition as the mechanism of action for NSAID. Semin Arthritis Rheum 1990;19:1-5.
77. Abramson S, Korchak HM, Kaplan H, et al. Modes of action of aspirin-like drugs. Proc Natl Acad Sci USA 1985;82:7227-7231.
78. Ford-Hutchinson AW. Modification of the lipoxygenase pathway of arachidonic acid metabolism. In Samuelsson B, Dahlen SE, Fritsch J, Hedqvist P eds. Advances in prostaglandin, thromboxane and leukotriene research. Trends in eicosanoid biology. New York Raven Press 1990:161-169.
79. Flower RJ. Steroidal anti-inflammatory drugs as inhibitors of phospholipase A2. In Galli C. Galli G, Porcelatti G. eds. Prostaglandins and phospholipases 1978;3:105-112
80. Hunter EJ. N-3 fatty acids from vegetable oils. Am J Clin Nutr 1990;51:809-814.

81. Lawson LD, Hughes BG. Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethylesters co-ingested with a high fat meal. Biochem Biophys Res Commun 1988;156:960-963.
82. Reicks M, Hoadley J, Satchithanandam S, Morehouse KM. Recovery of fish oil-derived fatty acids in lymph of thoracic duct-cannulated Wistar rats. Lipids 1990;25:6-10.
83. Lawson D, Hughes BG. Human absorption of fish oil fatty acids as triacylglycerols, free fatty acids, or ethyl esters. Biochem Biophys Res Comm 1988;152:328-335.
84. Hamazaki T, Urakaze M, Makuta M, et al. Intake of different eicosapentaenoic acid contaning lipids and fatty acid pattern of plasma lipids in the rats. Lipids 1987;22:994-998.
85. Blonk MC, Bilo HJ, Nauta JJ, Popp-Snijders C, Mulder C, Donker AJ. Dose-response effects of fish-oil supplementation in healthy volunteers. Am J Clin Nutr 1990;52:120-127.
86. Harris WS, Connor WE, McMurry MP. The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsturated fats: Salmon oil versus Vegetable oils. Metabolism 1983;2:179-184.
87. Goodnight SH, Harris WS, Connor WE. The effects of dietary omega-3 fatty acids on platelet composition and function in man: A prospective, controlled study. Blood 1981;5:880-885.
88. Schacky von C, Fischer S, Weber PC. Long-term of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. J Clin Invest 1985;76:1626-1631.
89. Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in eskimos. Lancet 1979;I:433-435.
90. Knapp HR, Reilly IAG, Alessandrini P, FitzGerald GA. In vivo indexes of platelet and vascular function during fish oil administration in patients with atherosclerosis. N Eng J Med 1986; 314:937-942.
91. Carwright IJ, Pockley AG, Galloway JH, Greaves M, Preston FE. The effects of dietary omega-3 polyunsaturated fatty acids on erythrocyte membrane phospholipids, eryhtrocyte deformability and blood viscosity in healthy volunteers. Atherosclerosis 1985;55:267-281.
92. Lee TH, Hoover RL, William JD, et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoid acids on in vitro neutrophil function. N Eng J Med 1985;312:1217-1223.
93. Endres S, Ghorbani R, Kelley VE, et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Eng J Med 1989;320:266-271.
94. Tempel vd H. Tulleken JE, Limburg PC, Muskiet FAJ, Rijswijk van MH. Effects of fish oil supplementation in rheumatoid arthritis. Ann Rheum Dis 1990;49:76-80.
95. Sperling RE, Weinblatt ME, Robin J-L, et al. Effects of dietary supplementations with marine fish oil on leukocyte lipid mediator generation and function in rheumatoid arthritis. Arthritis Rheum 1987;30:988-997.
96. Arm JP, Horton CE, Menica-Huerta JM, House F, Eiser NM, Clark TJH, Spur BW, Lee TH. Effect of dietary supplementation with fish oil lipids on mild asthma. Thorax 1988;43:84-92.
97. Schacky von C, Weber PC. Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoid acids in humans. J Clin Invest 1985;76:2446-2450.
98. Lorenz R, Sprengler U, Fischer S, Duhm J, Weber PC. Platelet function, thromboxane formation and blood pressure control during supplementation of the western diet with cod liver oil. Circulation 1983;67:504-511.
99. Schacky von C, Fisher S, Weber P. Long term effects of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. J Clin Invest 1985;76:1626-1631.
100. Hagve TA. Effects of unsaturated fatty acids on cell membrane functions. Scan J Clin Lab Invest 1988;48,381-388.
101. Garg ML, Thomson ABR, Clandinin MT. Effect of dietary cholesterol and/or omega-3 fatty acids on lipid composition and delta-5 desaturase activity of rat liver microsomes. J Nutr 1988;118:661-668.

102. Garg ML, Sebokova E, Thomson ABR, Clandinin MT. delta-6 Desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or n-3 fatty acids. Biochem J 1988;249:351-356.
103. Kinsella JE, Lokesh B, Stone RA: Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am J Clin Nutr 1990;52:1-28.
104. Lee TH, Hoover RL, Williams JD et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 1985;312:1217-1224.
105. Hornstra G, Haddeman E, Kloeze J, Verschuren PM. Dietary-fat-induced changes in the formation of prostanoids of the 2 and 3 series in relation to arterial thrombosis (rat) and atherosclerosis (rabbit). In Samelsson B, Paoletti R, Ramwell PW. eds In Adv Prostaglandin Thromboxane Leukotriene Res, New York Raven Press 1983;12:193-202.
106. Mehta JL, Lopez LM, Lawson D, Wargovich TJ, Williams LL. Dietary supplementation with omega-3 polyunsaturated fatty acids in patients with stable coronary heart disease. Am J Med 1988;84:45-51.
107. Needleman P, Raz A, Minkes MS, Ferrendelli JA, Sprecher H. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. Proc Natl Acad Sci USA 1979;76:944-948.
108. Knapp HR, Fitzgerald GA. The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. N Eng J Med 1989;320:1037-1043.
109. Payan DG, Wong MYS, Chernov-Rogan T, et al. Alterations in human leukocyte function induced by ingestion of eicosapentaenoic acid. J Clin Immunol 1986;6:402-410.
110. Prickett JD, Trentham DE, Robinson DR. Dietary fish oil augments the induction of arthritis in rats immunized with type II collagen. J Immunol 1984;132:725-728.
111. Lee TH, Mencia-Huerta JM, Shih C, Corey EJ, Lewis RA, Austen KF. Characterization and biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoid acid, including leukotriene B5 and the double lipoxygenase product. J Biol Chem 1984;259:2383-2389.
112. Lee TH, Mencia-Huerta J-M, Shih C, Corey EJ, Lewis RA, Austen KF. Effects of exogenous arachidonic eicosapentaenoid and docosahexaenoid acids on the generation of 5-lipoxygenase pathway product by ionophore-activated human neutrophils. J Clin Invest 1984;74:1922-1933.
113. Lee TH, Sethi T, Crea AGC, et al. Characterization of leukotriene B3: comparison of its biological activities with leukotriene B4 and leukotriene B5 in complement receptor enhancement, lysozyme release and chemotaxis of human neutrophils. Clin Science 1988;74:467-475.
114. Terano T, Salmon JA, Moncada S. Biosynthesis and biological activity of leukotriene B5. Prostaglandins 1984;27:217-232.
115. Leitch AG, Lee TH, Ringel EW, et al. Immunologically induced generation of tetraene and pentaene leukotrienes in the peritoneal cavities of menhaden-fed rats. J Immunol 1984;132:2559-2565.
116. Croft KD, Codde JP, Barden A, Vandongen R, Beillin L. Effect of dietary fish oils on the formation of leukotriene B4 and B5, thromboxane and platelet activating factor by rat leucocytes. Clin Exp Pharmacol Physiol 1988,15:517-525.
117. Meghi S, Sandy JR, Scutt AM, Harvey W, Harris M. Stimulation of bone resorption by lipoxygenase metabolites of arachidonic acid. Prostaglandins 1988;36:139-148.
118. Mohr W, Westerhellweg H, Wessinghage D. Polymorphonuclear granulocytes in rheumatic tissue destruction. III. An electron microscopic study of PMNs at the pannus-cartilage junction in rheumatoid arthritis. Ann Rheum Dis 1981;40:396-399.
119. Elmgreen J, Nielsen OH Ahnfelt-Ronne I. Enhanced capacity for release of leucotriene B4 by neutrophils in rheumatoid arthritis. Ann Rheum Dis 1987;46:501-505.
120. Leslie CA, Conte JM, Hayes KC, Cathcart ES. A fish oil diet reduces the severity of collagen induced arthritis after onset of the disease. Clin Exp Immunol 1988;73:328-332.
121. Cathcart ES, Mortensen RF, Leslie CA, Conte JM, Gonnerman WA. A fish oil diet inhibits amyloid P component (AP) acute phase responses in arthritis suspectable mice. J Immunol 1987;139:89-91.

122. McColl SR, Cleland LG, Whitehouse MW. Effect of dietary polyunsaturated fatty acid (PUFA) supplementation on adjuvant induced polyarthritis in rats. J Rheumatol 1987;14:197-201.
123. Kremer JM, Michalek AV, Lininger L, et al. Effects of manipulation of dietary fatty acids on clinical manifestations of rheumatoid arthritis. Lancet 1985;26:184-187.
124. Pike MC. Antiinflammatory effects of dietary lipid modification. J Rheumatology 1989;16:718-720.

Chapter 2

EFFECTS OF FISH OIL SUPPLEMENTATION IN RHEUMATOID ARTHRITIS.

Hille vd Tempel, Jacob E. Tulleken, Pieter C. Limburg,
Frits A.J. Muskiet, Martin H. van Rijswijk.

SUMMARY

Sixteen patients with rheumatoid arthritis entered a trial to determine the clinical and laboratory effects of dietary supplementation with fractionated fish oil fatty acids. We used a randomized, double blind, placebo-controlled crossover design with twelve week treatment periods. Treatment with non steroidal anti inflammatory drugs as well as with disease modifying anti-rheumatic drugs was continued throughout the study period. Placebo consisted of fractionated coconut oil. The following results favoured fish oil over placebo: joint swelling index ($p<0.05$) and duration of early morning stiffness ($p=0.01$). Other clinical parameters improved but did not reach statistical significance. During fish oil supplementation relative amounts of eicosapentaenoic acid and docosahexaenoic acid in the plasma cholesterol ester and neutrophil membrane phospholipid fractions increased, mainly at the expense of the omega-6 fatty acids. The mean neutrophil leukotriene B4 production in vitro showed a reduction after twelve weeks of fish oil supplementation. ($p<0.05$). Leukotriene B5 production, which could neither be detected in the control- nor in the placebo period, rose to substantial quantities during fish oil treatment. This study shows that dietary fish oil supplementation is effective in suppressing clinical symptoms of rheumatoid arthritis.

INTRODUCTION

Polyunsaturated fatty acids (PUFA) play an important role in the structural and metabolic function of cellular membranes. One of the major PUFA's is arachidonic acid. Oxygenation of arachidonic acid leads to the production of potent mediators of inflammation[1-3]. Alteration of dietary fatty acid composition may result in competition with the incorporation of arachidonic acid into the cell membrane and thereby modulate the functional qualities of the cell. Fatty acids commonly consumed in the western diet, e.g. linoleic acid, are converted via arachidonic acid into cyclooxygenase metabolites of the 2 series whereas leukotrienes of the 4 series are produced via the lipoxygenase pathway. Leukotrienes are potent lipid mediators which play an important role in allergic and inflammatory reactions[4-6]. Leukotriene B4 (LTB4), mainly produced in neutrophils, is a strong leukocyte activator. Its effects are comparable with C5a, F-Met-Leu-Phe and platelet activating factor and include chemotaxis, non-directed migration, aggregation, and lysosomal enzyme release[7-12].

PUFA with three carbon atoms from the terminal methyl group to the first double bond are designated omega-3 or n-3 fatty acids. Eicosapentaenoic acid (EPA; 20:5, n-3) and docosahexaenoic acid (DHA; 22:6,n-3) are the main long chain n-3 fatty acids in fish oil. Cell membrane incorporated 20:5,n-3 can be converted, via the cyclo-oxygenase pathway into prostaglandins, thromboxanes and prostacyclins of the 3 series. Via the lipoxygenase pathway leukotrienes of the 5-series, in particular leukotriene B5 (LTB5), are produced[13-15]. LTB5 is ten to thirty fold less potent than LTB4 in assays of leukocyte function[16,17]. Thus, dietary fish oil supplementation has potential anti-inflammatory effects, owing to the production of functionally attenuated 20:5,n-3 derived analogues of the arachidonic acid metabolites[18-21]. In this paper we describe the clinical and laboratory effects of dietary fatty acid supplementation with fish oil in patients with rheumatoid arthritis (RA).

PATIENTS AND METHODS

Patients:

Sixteen patients (9 females and 7 males, mean age 53 years, mean duration of disease 12 years) with RA were admitted to the study. Fifteen of them received non steroidal antiinflammatory drugs (NSAID). Eleven patients received 'disease modifying' anti-rheumatic drugs (DMARD) including gold salts, anti-malarials, and d-penicillamine in addition. None of the patients received steroids or cytostatic drugs.

Study Design:

We used a double blind, placebo-controlled, crossover design with twelve week treatment periods. Patients were randomly allocated to receive either 12 capsules of fractionated fish oil or fractionated coconut oil, flavoured with fish aroma, as placebo. (Intradal, Amersfoort, The Netherlands) per day. The fish oil capsules hold a majority of 20:5,n-3 (31 mol%) and 22:6,n-3 (22 mol%), resulting in a daily supply of 2.04 g 20:5,n-3 and 1.32 g 22:6,n-3, whereas the coconut oil capsules mainly contained 8:0 (63 mol%) and 10:0 (36 mol%). A twelve weeks run-in period without fatty acid supplementation was used to confirm stable disease activity. At week thirteen supplementation with either fish oil or placebo started. After twelve weeks the patients crossed over from fish oil to placebo or vice versa. Patients and physicians were blinded to treatment assignment during the entire study. The patients were instructed to continue their regular drug treatment schedule. Patient's dietary fat intake was kept constant throughout the study period. Each patient kept a daily food diary. Every two weeks the patients were observed by the same rheumatologist, biometrist and dietician. The study was approved by the local Medical Ethical Committee and informed consent was obtained from all patients.

Methods:

Clinical evaluation:

The clinical evaluation consisted of monitoring of: duration of early morning stiffness (in minutes), joint pain (joint pain index; on a four point scale 0=absent, 1=mild, 2=moderate, 3=severe) and joint swelling, using a three point scale (joint swelling index; 0=absent, 1=moderate, 2=severe), visual analogue pain scale (VAS) on a 10

point scale (from 0=no pain to 10=worst ever). Gripstrength was measured using a manometer (kPa).

Laboratory evaluation:
The laboratory evaluation of peripheral blood parameters was performed every two weeks and comprised a complete blood cell count, erythrocyte sedimentation rate (ESR) and plasma levels of C-reactive protein (CRP), fibrinogen, serum amyloid A (SAA), and IgM rheumatoid factor (IgM-Rf).

Assessment of in vitro production of leukotrienes:
Neutrophils were isolated from 30 ml peripheral blood drawn into EDTA. Erythrocytes were sedimentated with gelatine 0.3 % (g/v). The buffy coat was separated on 0.3% (g/v) gelatine and neutrophils were subsequently isolated on percoll (d=1.077 g/ml). Residual erythrocytes were lysed with hypotonic ammonium chloride. Neutrophils (10^7 cells/ml) were preincubated in Dulbecco's medium for 30 minutes at 37°. Subsequently calcium chloride was added to a final concentration of 2mM and the cells were stimulated with 10 uM calciumionophore A-23187 for 10 additional minutes, directly followed by centrifugation of the samples at 11,000 g for one minute. Finally Prostaglandin B2 was added to the supernatant as an internal standard for HPLC-measurements. Leukotrienes were separated and quantified using an C18 column[22].

Determination of oil supplement fatty acid composition:
Analyses of both medium and long chain fatty acids in the fish oil and coconut oil capsules were performed by a capillary gas chromatographic quantification technique, as described previously[23].

Determination of plasma cholesterol ester fatty acid composition:
After preparation of a plasma total lipid extract and isolation of the cholesterol ester fraction by amino propyl-silica columns, its fatty acid composition was determined by capillary gas chromatography[24].

Determination of neutrophil phospholipid fatty acid composition:
Neutrophil phospholipids were extracted with chloroform/methanol. Phospholipid subclasses were isolated by HPLC, essentially as described previously[25,26]. Their fatty acid composition was determined by capillary gas chromatography[27] using an apolar stationary phase.

Statistical analyses:
Data were analyzed using the standard methods based on the Student t-test[28]. For basic treatment comparisons the randomization p-values were calculated[29]. In order to assure that the overall significance level did not exceed 5% (one sided) the Bonferroni method was applied to correct for multiple tests. The p-value for each of the response variables was considered significant if less than 1% (one sided). We also calculated 90% confidence limits for mean treatment effects: based on the conventional t-test and without the Bonferroni correction. Relations between the response variables

and also between treatment effects on these variables were studied by means of the Spearman correlation coefficient.

RESULTS

Sixteen patients entered the trial. Two patients had to discontinue the study, one because of severe headaches during fish oil supplementation, the second because of a gastro-intestinal bleeding owing to an acenocoumarin overdosage. During both placebo treatment and during fish oil treatment only minor, short term side effects were observed (nausea, ructus and diarrhea). Most patients endured the fatty acid supplementation without any problems. During the run-in period no significant changes in clinical or laboratory parameters of disease activity were found. Since there was no washout period between the two 3-months treatment periods, the data were analyzed to determine whether there were treatment sequence effects. A "hangover" effect was perceived in most clinical and some laboratory variables at four weeks after cessation of fish oil administration. Yet at treatment endpoints the changes in clinical and laboratory variables appeared to be independent of treatment sequence.

Clinical evaluation:

Comparisons were made between endpoints of fish oil and placebo treatment. The differences are shown in Table 1. We observed a mean decrease of 6 points in the joint swelling index ($p<0.05$) after 12 weeks of fish oil treatment. The duration of morning stiffness decreased by a mean of 35 minutes ($p=0.01$) during fish oil treatment. The joint pain index showed a trend towards improvement during fish oil supplementation but did not reach statistical significance. Gripstrength and VAS were not altered.

Table 1 Clinical and laboratory disease variables before and after three months of fish oil and coconut oil treatment. Values are expressed as mean(SEM), unless otherwise indicated.

	Supplementation		
	None	Fish oil	Coconut oil
Disease variables			
Joint pain index (pnts)	33.0 (7.0)	29.0 (7.0)	42.0 (9.0)
Joint swelling index (pnts)	10.0 (2.0)	2.0 (1.0)*	8.0 (3.0)
Morning stiffness (min)	46.0 (15.0)	15.0 (5.0)**	50.0 (13.0)
VAS[a]	3.1 (0.6)	2.7 (0.5)	4.0 (0.7)
CRP (mg/l)	30.0 (3.0-121)	17.0 (3.0-69)	21.0 (5.0-71)
SAA (mg/l)$	14.0 (1.0-100)	14.0 (1.0-127)	28.0 (1.0-95)
Fibrinogen (g/l)$	4.4 (2.6-7.3)	4.3 (2.4-5.6)	4.3 (2.9-5.3)
ESR (min)$	30.0 (19.0-98)	34.0 (14.0-80)	40.0 (11.0-70)
IgM-Rf (IU/ml)$	130.0 (3.0-1050)	100.0 (3.0-850)	165.0 (3.0-900)

a: visual analogue pain scale. $: median (range); * $p=0.01$, one sided, significantly different from coconut oil and pre diet values. 90% confidence limits= 1-11 for mean treatment effect. ** $p<0.05$, one sided, significantly different from coconut oil and pre diet values. 90% confidence limits= 14-55 for mean treatment effect.

Laboratory evaluation:
Median(range) levels at baseline, after twelve weeks of fish oil or placebo supplementation of plasma fibrinogen, CRP, SAA, IgM-Rf and of ESR are also shown in Table 1. The complete blood cell count was not significantly altered. The differences between neutrophil LTB4 production at baseline and after 12 weeks of fish oil and coconut oil supplementation are shown in Table 2. Mean values decreased from a base line production of 149 ng/10^7 neutrophils to 123 ng/10^7 neutrophils ($p<0.05$) and LTB5 production increased from undetectable amounts to a mean production of 13 ng/10^7 neutrophils ($p<0.01$). Changes in clinical variables did not correlate with changes in the leukotriene production.

Table 2 In vitro leukotriene production (ng/10^7 neutrophils) by neutrophils isolated from the blood of patients with RA before and after three months of dietary fatty acid supplementation with fish oil or coconut oil. mean(sem).

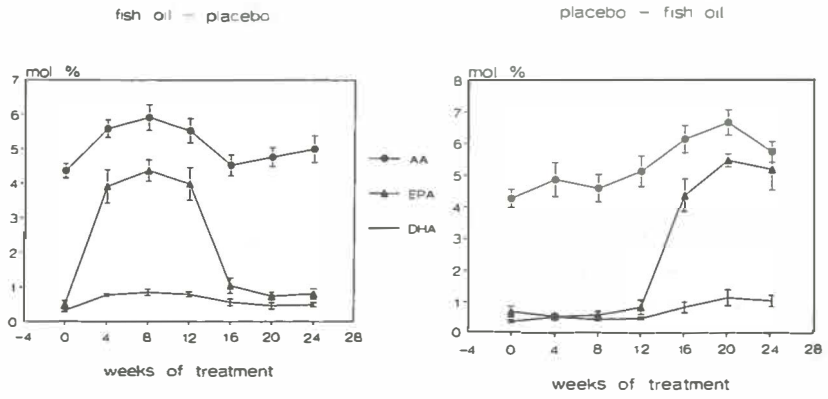
	Supplementation		
	None	Fish oil	Coconut oil
LTB4	149.0 (13.0)	123.0 (10.0)*	141.0 (12.0)
LTB5	0.0 (0.0)	13.0 (2.0)**	0.0 (0.0)

* $p<0.05$, one sided, significantly different from base line values. 90% confidence limits = 6-46 for mean treatment effect.
** $p<0.01$, one sided, significantly different from base line and coconut oil values. 90% confidence limits = 9-17 for mean treatment effect.

Within eight weeks after discontinuation of fish oil supplementation LTB5 production had declined below detection level again.

Plasma cholesterol esters and neutrophil membrane phospholipids:
In the placebo period, no significant alterations were observed in the fatty acid composition of the plasma cholesterol ester fraction. The course of the composition for selected fatty acids in the plasma cholesterol ester fraction during the study is shown in Figure 1. The n-3 fatty acid incorporation did reach a maximum within

Figure 1 Treatment sequence effects (fish oil-placebo and placebo-fish oil) on the relative composition of selected fatty acids in the plasma cholesterol ester fraction.

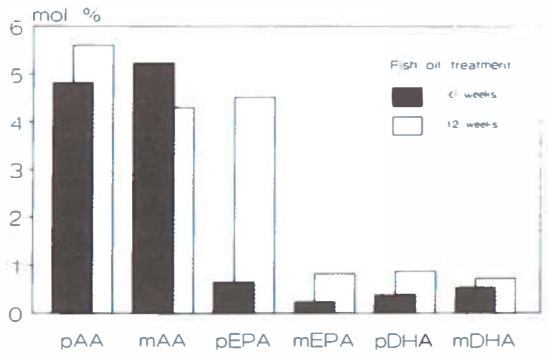


Patients crossed over after twelve weeks. Values are expressed as mean(sem).

one month of fish oil administration. In all cases these values persisted during the fish oil period. In the plasma cholesterol ester fraction the n-3 fatty acids replaced notably the n-6 fatty acids: linoleic acid (18:2,n-6), gamma-linolenic acid (18:3,n-6) and di-homo-gamma-linolenic acid (20:3,n-6). However, the amount of arachidonic acid (20:4,n-6) significantly increased.

Figure 2 demonstrates the differences between incorporation of relative amounts of selected fatty acids in the plasma cholesterol ester fraction and in the total phospholipid fraction of isolated neutrophils before and during twelve weeks of fish oil supplementation. Owing to technical problems the fatty acid composition of neutrophil membrane phospholipids was calculated for only nine patients In both fractions 20:5,n-3 was significantly incorporated ($p<0.01$). In contrast with the increase of 22:6,n-3 in the plasma cholesterol ester fraction ($p<0.01$), the increment of relative amounts of 22:6,n-3 in the neutrophil membrane phopholipids during fish oil supplementation was not statistically significant. The 20:4,n-6 content in the neutrophil membrane phospholipids was also not significantly altered during fish oil supplementation.

Figure 2 Differences between incorporation of relative amounts (mol%) of selected fatty acids in the plasma cholesterol ester fraction and in the total phospholipid fraction isolated from neutrophils before and after three months of fish oil supplementation. Values are expressed as mean.



p = plasma cholesterol ester fraction; m = neutrophil membrane phospholipid fraction; AA = Arachidonic acid (20:4,n-6); EPA = Eicosapentaenoic acid (20:5,n-3); DHA=Docosahexaenoic acid (22:6,n-3)

DISCUSSION

The management of patients with RA is difficult. A variety of drugs, either alone or in combination are used with varying success to suppress symptoms or modify disease activity. In extensively treated patients, the number of side effects is considerable and may disturb treatment. Physicians and patients have long been intrigued by the notion that some foods or food related products can provoke or alleviate rheumatic symptoms[30-35]. It is also known that fasting has an antiinflammatory effect in RA[36,37]. A more sophisticated approach is based on the fact that alteration of the dietary fatty acid composition has been shown to modulate the production of eicosanoids[13-20,38,39].

Our study demonstrates significant improvement of clinical disease variables in patients with RA during dietary fish oil supplementation. Although statistically significant improvement was only achieved for joint swelling index and duration of early morning stiffness, most clinical variables favoured fish oil over placebo. Based on subjective clinical observations it can be estimated that most patients experienced considerable relief on fish oil supplementation. Even more impressive was the improvement of patients assessment of disease activity. A readily available method to assess subjective improvement, the visual analogue pain scale, did however not demonstrate statistically significant alterations. Subjective improvements were also observed during coconut oil treatment, therefore we conclude that the subjective alleviations are at least partially due to a placebo effect.

In contrast to the clinical disease variables, the changes in the laboratory parameters of inflammation were not statistically significant. Our findings are in agreement with the results of other clinical studies on dietary n-3 fatty acid supplementation in RA[20,38,39]. However a recent report[40] showed a significant decrease of mean plasma fibrinogen levels during a six weeks daily supply of 14 g fish oil (3.5 g 20:5,n-3,) in healthy volunteers. In our trial 6 g fish oil (2.04 g 20:5,n-3) has no influence of statistical importance neither on the mean plasma fibrinogen concentration nor on the plasma levels of other acute phase proteins like CRP and SAA. A possible explanation for these apparently conflicting findings might be a dose dependent effect of fish oil on fibrinogen synthesis.

In spite of the lack of alterations in the levels of acute phase proteins, fish oil supplementation results in changes in the leukotriene production of in vitro stimulated neutrophils. A small production of LTB5 was observed, concomitant with a decrease of the mean LTB4 production. The slight production of LTB5 is in accordance with the small amounts of 20:5,n-3 incorporated in the total phospholipid fraction from isolated neutrophils. A major question regarding the leukotrienes is whether any of the induced alterations contribute to the result of clinical improvement during fish oil administration. Since no correlations were found between changes in the leukotriene production and the significantly altered disease variables, it seems unlikely that inhibition of the lipoxygenase pathway is the only cause of clinical improvement. Accordingly to the pharmacologic potency of fish oil to modulate the formation of leukotrienes, prostacyclin and prostaglandins, it should be considered that the effects of fish oil may be the result of changes in the production of prostaglandins. In this context it is interesting to note that even during the use of drugs that block prostaglandin synthesis (NSAID) fish oil provides an additive beneficial effect.

In conclusion this study shows that administration of n-3 fatty acids is effective in suppressing clinical symptoms of RA. Convincing biochemical evidence that n-3 fatty acids may also act as a DMARD could not be obtained, however. Although a change in neutrophil leukotriene production in vitro could be clearly shown, the question remains whether the clinical effects can be accounted for by inhibition of the lipoxygenase pathway.

ACKNOWLEDGEMENTS

The authors wish to thank W. Wassenaar for nursing assistance, M. Strubbe and I. Boomgaardt for dietary assistance, K. Kazemier, G Meiborg, H.M. Rutgers, I.A. Martini for their valuable technical assistance, drs. M.A. van Leeuwen and dr. R.L.F. Nienhuis, rheumatologists, for medical advice.

REFERENCES

1. Borgeat P, Samuelsson B. Arachidonic acid metabolism in polymorponuclear leukocytes: effects of ionophore A23187. Proc Natl Sci USA 1979;5:2148-2152.
2. Zijlstra FJ, Naaktgeboren M, Mons H, Vincent JE. Formation of prostaglandins and leukotrienes by human lung tissue in vitro after activation by the calcium ionophore A23187. Eur J Clin Invest 1987;17:325-329.
3. Malmsten CL. Prostaglandins, thromboxanes, and leukotrienes in inflammation. Semin Arthritis Rheum 1985;2:29-35.
4. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxines: Structures, biosynthesis, and biological effects. Science 1987;237:1171-1175.
5. Samuelsson B. Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammation. Science 1983;220:568-575.
6. Parker CW. 5-Lipoxygenase, leukotrienes and regulation of inflammatory responses. Drug Dev Res 1987;10:277-293.
7. Klickstein LB, Shapleigh C, Goetzl EJ. Lipoxygenation of arachidonic acid as a source of polymorphonuclear leukocyte chemotactic factors in synovial fluid and tissue in rheumatoid arthritis and spondyloarthritis. J Clin Invest 1980;66:1166-1170.
8. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJH. Leukotriene B, a chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 1980;286:264-265.
9. Elmgreen J, Nielsen OH, Ahnfelt-Ronne I. Enhanced capacity for release of leukotriene B4 by neutrophils in rheumatoid arthritis. Ann Rheum Dis 1987;46:501-505.
10. Hoover RL, Karnovsky MJ, Austen KF, Corey EJ, Lewis RA. Leukotriene B4 action on endothelium mediates augmented neutrophil/endothelial adhesion. Proc Natl Acad Sci USA 1984:2191-2193.
11. Palmer RMJ, Stepney RJ, Higgs GA, Eakins KE. Chemokinetic activity of arachidonic acid lipoxygenase products on leukocytes of different species. Prostaglandins 1980;2:411-418.
12. Bray MA, Ford-Hutchinson AW, Smith MJH. Leukotriene B4: an inflammatory mediator in vivo. Prostaglandins 1981;22:213-222.
13. Higgs GA. The effects of dietary intake of essential fatty acids on prostaglandin and leukotriene synthesis. Proc Nutr Soc 1985;44:181-187.
14. Kelley VE, Ferretti A, Izui S, Strom TB. A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites, and suppresses lupus in mrl-lpr mice. J. Immunol 1985;3:1914-1919.
15. Strasser T, Fisher S, Weber PC. Leukotriene B5 is formed in human neutrophils after dietary supplementation with icosapentaenoic acid. Proc Natl Acad Sci USA 1985;82:1540-1543.
16. Terano T, Salmon JA, Moncado S. Biosynthesis and biological activity of leukotriene B5. Prostaglandins 1984;27:217-232.
17. Lee TH, Mencia-Huerta JM, Shih C, Corey EJ, Lewis RA, Austen KF. Characterization and biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B5 and the double lipoxygenase product. J Biol Chem 1984;4:2383-2389.
18. Lee TH, Hoover RL, Williams JD, et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 1985;19:1217-1221.
19. Payan DG, Wong MYS, Chernov-Rogan T, et al. Alterations in human leukocyte function induced by ingestion of eicosapentaenoic acid. J Clin Immunol 1986;5:402-410.

20. Sperling I, Weinblatt M, Robin JL, et al. Effects of dietary supplementation with marine fish oil on leukocyte lipid mediator generation and function in rheumatoid arthritis. Arthritis Rheum;30:988-997.
21. Leslie CA, Conte JM, Hayes KC, Cathcart ES. A fish oil diet reduces the severity of collagen induced arthritis after onset of the disease. Clin Exp Immunol 1988;73:328-332.
22. Verhagen J, Walstra P, Veldink GA, Vliegenthart JFG, Bruynzeel PLB. Separation and quantitation of leukotrienes by reversed phase high performance liquid chromatography. Prostaglandins Leukotrienes Med 1984;13:15-20.
23. Steege van der G, Muskiet FAJ, Martini IA, Hutter NH, Boersma ER. Simultaneous quantification of total medium- and long chain fatty acids in human milk by capillary gas chromatography with split injection. J Chromatogr Biomed Appl 1987;415:1-11.
24. Hoving EB, Jansen G, Volmer M, Doormaal van JJ, Muskiet FAJ. Profiling of plasma cholesterol ester and triglycerid fatty acids as their methyl esters by capillary gas chromatography, preceded by a separation of lipid classes. J Chromatogr Biomed Appl 1988;434:395-409.
25. Zijlstra JG, Vries de JG, Muskiet FAJ, Martini IA, Timmer-Boscha H, Mulder NH. Influence of docosahexaenoic acid in vitro on intracellular adriamycin-sensitive and resistant small-cell lung cancer cell lines, and on cytotoxicity in the tumor cell lines. Int J Cancer 1987;40:850-856.
26. Hoving EB, Prins J, Rutgers HM, Muskiet FAJ. Behaviour of plasmalogens during high performance liquid chromatography on a silica collumn and a phosphoric acid containing mobile phase. J Chromatogr Biomed Appl 1988;434:411-416.
27. Muskiet FAJ, Doormaal van JJ, Martini IA, Wolthers BG, Slik van der W. Capillary gas chromatographic profiling of total long chain fatty acids and cholesterol in biological materials. J Chromatogr Biomed Appl 1983;278:231-244
28. Pocock SJ, Clinical trial, Wiley 1983;110-122,29. Edginton ES, Randomisation tests, Marcel Dekker 1980;101-130.
30. Anonymous. Fish oils in rheumatoid arthritis. Lancet 1987;I:720-721.
31. Panush RS, Stroud RM, Webster EM. Food induced (allergic) arthritis, inflammatory arthritis exacerbated by milk. Arthritis Rheum 1986;2:220-226.
32. Beri D, Malaviya AN, Shandilya R, Singh RR. Effect of dietary restrictions on disease activity in rheumatoid arthritis. Ann Rheum Dis 1988;47:69-72.
33. Ziff M. Diet in the treatment of rheumatoid arthritis. Arthritis Rheum 1983;4:457-471.
34. Anonymous. Nutritional therapy for rheumatic diseases. Ann Intern Med 1987;4:619-620.
35. Parje AL, Hughes GRV. Rheumatoid arthritis and food. Br Med J 1981;282:2027-2029.
36. Hafstrom I, Ringertz B, Gyllenhammar H, Palmblad J, Harms-Ringdal M. Effects of fasting on disease activity, neutrophil function, fatty acid composition, and leukotriene biosynthesis in patients with rheumatoid arthritis. Arthritis Rheum 1988;5:585-592.
37. Uden AM, Trang L, Venizelos N, Palmblad J. Neutrophil functions and clinical performance after total fasting in patients with rheumatoid arthritis. Ann Rheum Dis 1983;42:45-51.
38. Kremer JM, Bigaouette J, Michalek AV, et al. Effects of manipulation of dietary fatty acids on clinical manifestations of rheumatoid arthritis. Lancet 1985;I:184-187.
39. Kremer JM, Jubiz W, Michalek AV, et al. Fish oil fatty acid supplementation in active rheumatoid arthritis. Ann Intern Med 1987;106:497-503.
40. Hostmark AT, Bjerkedal T, Kierulf P, Flaten H, Ulshagen K. Fish oil and plasma fibrinogen. Br Med J 1988;297:180.

Reproduced with permission from the Annals of the Rheumatic Diseases

Chapter 2a

OMEGA-3 POLYUNSATURATED FATTY ACIDS INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR

Jacob E Tulleken, Pieter C Limburg, Martin H van Rijswijk

LETTER TO THE EDITOR

Dear Sir,

Dr. Endres and coworkers assigned nine healthy volunteers to receive 18 g fish oil concentrate (2.8 g eicosapentaenoic acid + 1.9 g docosahexaenoic acid)[1]. They found a significant suppression of interleukin-1 (IL-1) and tumor necrosis factor (TNF) produced *in vitro* by stimulated peripheral blood mononuclear cells. Suppression of IL-1 and TNF persisted even for as long as 10 weeks after cessation of dietary omega-3 (n-3) supplementation. This observation is an important addition to the evidence that n-3 polyunsaturated fatty acids may be of benefit in inflammatory conditions, like rheumatoid arthritis.

The authors suggest that the decreased IL-1 production might be the result of decreased synthesis of leukotriene B4 and the generation of the fish oil derived, almost inactive, metabolite leukotriene B5. Unfortunately, dr Endres and colleagues did not report alterations in leukotriene production. Recently we studied the clinical and biochemical effects of dietary n-3 polyunsaturated fatty acid administration in patients with rheumatoid arthritis[2]. In our double blind placebo controlled, crossover trial with 12 weeks treatment periods, 16 patients with active though stable rheumatoid arthritis (American Rheumatism Association criteria) were randomly allocated to receive 2.04 g eicosapentaenoic acid and 1.32 g docosahexaenoic acid or placebo (coconut oil) administered in 12 capsules daily. Fish oil produced a statistically significant improvement in clinical disease variables but the biochemical measures of disease activity remained unchanged. Furthermore suppression of the generation of leukotriene B4, by *in vitro* stimulated neutrophils with calcium ionophore following fish oil administration was observed whereas small quantities of leukotriene B5 became detectable Within eight weeks after cessation of fish oil administration leukotriene B4 and leukotriene B5 formation were returned to pre-diet production levels (Table 1).

Table 1 In vitro production of leukotriene B4 and leukotriene B5 by isolated neutrophils of 14 patients with rheumatoid arthritis during dietary fish oil supplementation.

Variable	Before n-3 supplement	After n-3 supplement	8 weeks after supplement
Leukotriene B4	149 (12)	123 (10)*	181 (13)
Leukotriene B5	0 (0)	13 (2)**	0 (0)

Values are expressed as ng/10^7 neutrophils. * $p<0.05$; ** $p<0.01$.

Our results indicate that a decreased IL-1 and TNF production at 10 weeks after cessation of fish oil administration are apparently not the result of alterations of 5-lipoxygenase metabolites. To our point of view an other at the moment unknown mechanism should be considered.

Since IL-1 is perceived to be one of the key mediators in rheumatoid arthritis decreased production of this cytokine as a result of dietary n-3 fatty acids supplementation may be of practical importance in the treatment of patients with rheumatoid arthritis. Thus the observations of the authors are worthy of further study.

REFERENCES

1. Endres S, Ghorbani R, Kelley VE et al. The effect of dietary supplementation with omega-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 1989;320:265-271.
2. Tempel vd H. Tulleken JE, Limburg PC, Muskiet FAJ, Rijswijk van MH. Effects of fish oil supplementation in rheumatoid arthritis. Ann Rheum Dis 1990;49:76-80.

Reproduced with permission from the New England Journal of Medicine

Chapter 3

VITAMIN E STATUS DURING DIETARY FISH OIL SUPPLEMENTATION IN RHEUMATOID ARTHRITIS

Jacob E. Tulleken, Pieter C. Limburg, Frits A.J. Muskiet,
Martin H. van Rijswijk

SUMMARY

The primary objective of this study was to determine whether it is the fish oil itself or the alpha-tocopherol that is added to the fish oil preparations (to prevent peroxidation) that is responsible for the beneficial effects of dietary supplementation with fish oil in patients with rheumatoid arthritis (RA). One group of RA patients took fish oil supplements and another group took alpha-tocopherol-enriched coconutoil supplements (placebo controls), both for 3 months. Clinical and laboratory indices of RA activity in relation to cellular and plasma vitamin E levels were assessed at the beginning and at the end of the trial. The results of the study provide evidence that the beneficial effects of fish oil supplementation cannot be ascribed to the anti-oxidizing properties of alpha-tocopherol perse.

INTRODUCTION

Dietary supplementation with fish oils, rich in omega-3 (n-3) polyunsaturated fatty acids (PUFA), may lead to a considerable relief of pain in patients who have rheumatoid arthritis (RA)[14]. The suggested mechanism involved is the enrichment of n-3 PUFA in the phospholipid fraction of cell membranes and the concomitant decrease in n-6 PUFA, thereby reducing the content of precursor for the 4-series leukotrienes and the 2-series prostaglandins. Fish oil preparations, however, are enriched in alpha-tocopherol, which prevents the peroxidation of PUFA. It is conceivable that the antioxidizing properties of alpha-tocopherol may interfere with the process of inflammation by reducing the free radical-mediated cellular lipid peroxidation.

Thus the question has been raised whether the antiinflammatory effects of fish oil are due to the action of n-3 PUFA or the alpha-tocopherol only. Therefore we compared the effects of dietary fish oil supplementation with the effects of alpha-tocopherol-enriched coconut oil supplementation in RA patients. Clinical disease variables were studied in relation to cellular and plasma vitamin E levels in these patients.

PATIENTS AND METHODS

Patients:
Twenty eight patients who met the American Rheumatism Association criteria for RA participated in the study. All but one had clinically active disease, as defined by the presence of at least 2 of the following criteria: duration of early morning stiffness

> 45 minutes, at least six tender joints, swelling in at least three joints and CRP>6 mg/l. Concurrent treatment for RA consisting of nonsteroidal anti-inflammatory and disease modifying anti-rheumatic drugs were required to be stable for at least three months prior to the study as well as during the study. Informed consent was obtained from all patients.

Methods:

Supplementation with fish oil and coconut oil/alpha-tocopherol:

The patients were randomly assigned by the pharmacy department of the hospital to receive either the fish oil supplement or the coconut oil supplement (placebo control group). The supplements were given as 12 identically appearing capsules/day for a total duration of three months. To prevent identification of the coconut oil fish flavour was added to the capsules. Both oil supplements were a gift from Aerofako, Amersfoort, The Netherlands. The fish oil capsules mainly contained 20:5,n-3 (31 mol%) and 22:6,n-3 (22 mol%) fatty acids (ethyl esters), resulting in a daily supply of 2.04 g 20:5,n-3 and 1.32 g 22:6,n-3. The total dose of the fish oil supplement on a daily basis for each patient was 6 g. The coconut oil capsules mainly contained 8:0 (63 mol%) and 10:0 (36 mol%) fatty acids[3]. The alpha-tocopherol content of the fish oil capsules was 5 micromol/ml oil (12.9 mg alpha-tocopherol daily) and that of the coconut oil capsules 4 micromol/ml oil (10.3 mg alpha-tocopherol daily). Alpha-tocopherol was the only tocopherol that was added to the capsules. Patients were asked to adhere to their dietary habbits. Each patient kept a daily food diary.

Clinical protocol:

Clinical evaluation consisted of: gripstrength of each hand (kPa); Ritchie articular index[5]; the total number of swollen and tender joints; duration of early morning stiffness (in minutes); pain score on a visual analogue scale VAS, 0-10, from no pain to worst ever). For the degree of tenderness a scale of 0-3 was used (joint pain index; (JPI) 0 = no tenderness, 1 = mild, 2 = moderate, 3 = severe) and a scale of 0-2 was used to assess the degree of swelling (joint swelling index; (JSI) 0 = none, 1 = mild, 2 = severe) of selected joints. The follow up time was three months with clinical check ups every month. Blood samples were collected during each visit.

Laboratory tests:

Standard laboratory tests were performed including a complete blood cell count, erythrocyte sedimentation rate (ESR), plasma C-reactive protein (CRP), IgM-rheumatoid factor (IgM-Rf), serum total cholesterol and triglycerides. Vitamin E levels in plasma, erythrocytes and capsules were determined by high performance liquid chromatography with fluorescent detection, essentially as described by others[6,7]. Plasma and erythrocyte alpha-tocopherol equivalent was calculated using the formula: alpha-tocopherol + (0.1 x gamma-tocopherol).

Statistical analysis:

The Mann-Whitney U test was used to compare the groups at baseline, after treatment and changes from baseline. p Values less than 0.05 were considered significant.

RESULTS

Table 1 shows that the two groups were similar in patient characteristics at study entry.

Table 1 Patient characteristics at study entry

	Fish oil	Control
Age (mean(range))*	52.0 (29.0-66.0)	58.0 (43.0-68.0)
Males/females	1:12	2:12
Duration of disease*	18.0 (6.0-30.0)	20.0 (3.0-48.0)
Current Medication**		
NSAID	12	12
Gold	-	1
d-Penicillamine	7	6
Anti-malarials agents	3	4
None	1	-

The clinical and laboratory indices of disease activity of both groups preceding the supplementation protocol are presented in Table 2. At study entry, plasma CRP and

Table 2 Clinical and laboratory indices of disease activity of patients with RA before and during a three month period of dietary fish oil or coconut oil/alpha-tocopherol supplementation. Median (range).

	Fish oil		Control		
	Before	After	Before	After	Inter group p
ESR (mm)	36.0 (8.0-74.0)	21.0 (7.0-60.0)	62.0 (2.0-82.0)	53.0 (6.0-86.0)	
CRP* (mg/l)	8.0 (3.0-54.0)	14.0 (1.0-52.0)	27.0 (4.0-110.0)	18.0 (3.0-101.0)	
IgM-Rf* (IU/ml)	40.0 (7.0-750.0)	43.0 (5.0-650.0)	135.0 (28-1500)	170.0 (38.0-1700)	
JPI	27.0 (3.0-103.0)	6.0 (0.0-49.0)	27.0 (5.0-52.0)	20.0 (4.0-48.0)	<0.05
Ritchie	18.0 (3.0-49.0)	6.0 (0.0-49.0)	15.5 (5.0-27.0)	11.5 (4.0-29.0)	<0.05
Pain (n)	18.0 (2.0-42.0)	8.0 (0.0-42.0)	21.0 (4.0-27.0)	14.0 (4.0-34.0)	
JSI	7.0 (0.0-26.0)	4.0 (1.0-16.0)	6.0 (2.0-14.0)	4.0 (1.0-16.0)	
Swelling (n)	6.0 (0.0-24.0)	3.0 (1.0-16.0)	5.0 (2.0-13.0)	4.0 (1.0-16.0)	<0.05
Gripstrength					
R(kPa)	0.2 (0.0-1.2)	0.3 (0.0-1.3)	0.2 (0.0-1.3)	0.2 (0.0-1.3)	
L(kPa)	0.3 (0.0-0.8)	0.4 (0.0-0.8)	0.2 (0.0-1.2)	0.2 (0.1-2.0)	
Stiffness (min)	60.0 (0.0-60.0)	30.0 (0.0-120)	45.0 (0-120.0)	60.0 (0.0-180)	
VAS	4.0 (0.5-6.1)	2.4 (0.0-7. 4)	4.4 (1.4-8.0)	3.8 (0.5-8.1)	

$ Intergroup p denotes the level of significance for the change from baseline in the fish oil group versus change from baseline in the placebo group. * $p<0.05$, significantly different for comparison of groups at baseline.

IgM-Rf levels were significantly lower in the fish oil group in comparison with the control group as a result of the randomization protocol. One patient in the fish oil group discontinued the supplementation study within one week of treatment, not tolerating the size and number of the capsules. Both supplements caused short term (days), mild gastro-intestinal discomforts (nausea, ructus, diarrhea). One patient developed a rash and pruritus during coconut oil supplementation.

Disease activity:

At the end of the study data for 13 fish oil and 14 coconut oil supplemented patients were available for evaluation. Although some clinical improvement ocurred in the

control group, the clinical improvement with fish oil was greater than that with coconut oil/alpha-tocopherol (see also Table 2). The laboratory indices of disease activity did not change in the fish oil group, nor in the control group.

Plasma and erythrocyte vitamin E status parameters:
Presupplementation values of vitamin E status parameters in plasma and erythrocytes were similar in both groups and were within the normal range. (Table 3)

Table 3 Vitamin E status parameters in patients with RA before and after a three month period of dietary fish oil or coconut oil/alpha-tocopherol supplementation.

Variable	Fish oil		Coconut oil		Intergroup P
	Before	After	Before	After	
PLASMA					
alpha-T	24.8 (21.6-35.0)	27.1 (21.2-53.6)	26.6 (16.2-44.6)	30.8 (18.1-49.4)	
gamma-T	3.9 (0.8-5.7)	2.8 (0.2-4.8)	2.8 (1.0-5.9)	2.6 (1.0-6.8)	
alpha-T eq	25.1 (21.9-35.4)	27.4 (21.7-54.1)	26.8 (16.5-44.9)	30.9 (18.4-49.7)	
alpha-T eq/tl	3.9 (2.6-4.9)	4.3 (2.6-7.3)	3.9 (2.6-5.4)	4.2 (3.5-6.8)*	
ERYTHROCYTES					
alpha-T	6.5 (4.1-9.8)	7.6 (6.1-8.9)	6.4 (4.7-7.5)	9.2 (6.2-10.9)*	<0.01
gamma-T	1.1 (0.1-1.9)	1.1 (0.7-2.0)	1.0 (0.4-1.9)	1.5 (0.7-1.7)**	
alpha-T eq	6.6 (4.2-9.9)	7.6 (6.1-9.0)	6.5 (4.8-7.5)	9.3 (6.3-11.0)*	<0.01

* $p<0.01$ and ** $p<0.05$ versus before coconutoil/alpha-tocopherol supplementation. Intergroup P for the between treatment group comparison of after treatment values in erythrocytes. T: tocopherol; alpha-T eq: alpha-tocopherol equivalent; tl=total lipid-(=serum cholesterol + triglycerides); For plasma alpha-T, gamma-T, alpha-T eq in micromol/l, plasma alpha-T eq/tl in mmol/mol and erythrocyte alpha-T, gamma-T and alpha-T eq in micromol/10^{13} erythrocytes.

Despite a slightly lower daily intake (2.6 mgr) of alpha-tocopherol in the control group the patients showed significant increases of several vitamin E status parameters when compared with the fish oil group (Table 3). In both groups no correlations were found between changes in disease activity and vitamin E status.

DISCUSSION

In controlled studies, we and others have previously reported that fish oil supplementation is effective in suppressing clinical symptoms in patients with active RA[1-4]. Concomitant with the clinical improvements there is a decreased production of the pro-inflammatory arachidonic acid derived metabolites and an increase of the functionally attenuated n-3 PUFA derived analogs. In general, alpha-tocopherol is added to the fish oil capsules to prevent unwanted lipid peroxidation. To our knowledge little or no attention has been given to whether it is the n-3 PUFA, alpha-tocopherol, or the combination of both that is responsible for the antiinflammatory effects of dietary fish oil supplementation. Therefore, the primary objective of this trial was to determine whether the fish oil or the alpha-tocopherol affects joint symptoms, by adding similar amounts of alpha-tocopherol to supplements given to both the experimental and the control group.

Consistent with the results of previous trials and despite a notable increase of the cellular vitamin E status parameters in the control group, the current study demonstrates that dietary supplementation with the fish oil is superior to alpha-tocopherol enriched coconut oil in producing clinical benefit in RA. This was most clear

with respect to the number of swollen joints, joint pain index and Ritchie articular index. Thus our data reinforce the conclusion that the n-3 PUFA, rather than the anti-oxidizing properties of the encapsuled alpha-tocopherol, are mainly responsible for the improvement of joint symptoms during fish oil supplementation in patients with RA. It should be emphasized, however, that a potentiating effect of the anti-oxidant on the anti-inflammatory effects of the n-3 PUFA can not be excluded.

Since the degree of unsaturation of cell membranes increases the risk for lipid peroxidation and the generation of toxic free radicals the alpha-tocopherol requirements depends on the amount of PUFA consumed. Recent studies, however, showed no changes in plasma parameters of vitamin E status during supplementation of alpha-tocopherol enriched fish oil in normal controls and hyperlipidemic patients[8,9]. In accordance with these studies, our data indicate that despite the substantially increased intake of n-3 PUFA, the amount of alpha-tocopherol added to the fish oil capsules was sufficient to prevent deficiencies in cellular and plasma levels of vitamin E. Unlike the situation in the control group, however, no statistically significant increase in vitamin E status parameters occurred in the fish oil treatment group. It remains to be determined whether this has to be explained by an increased expenditure of alpha-tocopherol due to peroxidation of n-3 PUFA in the tissues.

In summary, this study confirms the beneficial clinical effect of dietary fish oil supplementation in patients with RA. This effect cannot be ascribed to the anti-oxidizing properties of alpha-tocopherol in the fish oil capsules per se.

ACKNOWLODGEMENTS

We are indebted to ms. I.K. Boomgaardt, ms K.M. Kazemier, mr. H.J.R. Velvis, ms. W.Wassenaar and ms. J. Wever.

REFERENCES

1. Kremer JM, Jubiz W, Michalek AV, et al. Fish oil fatty acid supplementation in active rheumatoid arthritis. Ann Intern Med 1987;106:497-503.
2. Sperling I, Weinblatt M, Robin JL, et al. Effects of dietary supplementation with marine fish oil on leukocyte lipid mediator generation and function in rheumatoid arthritis. Arthritis Rheum 1987;30:988-997.
3. Tempel vd H, Tulleken JE, Limburg PC, Muskiet FAJ, Rijswijk van MH. Effects of fish oil supplementation in rheumatoid arthritis. Ann Rheum Dis, 1990;49:76-80.
4. Cleland LG, French JK, Betts WH, Murphy GA, Elliot MJ. Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. J Rheumatol 1988;15:1471-1475.
5. Ritchie DM, Boyle JA, McInnes JM, et al. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. Quart J Med 1968;37:393-406.
6. Leenheer de AP, Bevere de VORC, Cleys AE. Measurement of alpha-, bèta- and gamma-tocopherols in serum by liquid chromatography. Clin Chem 1979;25:425-428.
7. Lehman J, Martin HL. Improved direct determination of alpha and gamma tocopherols in plasma and platelets by liquid chromatography with fluorescence detection. Clin Chem 1982;28:1784-1787.
8. Knapp HR, Reilly IAG, Alessandrini P, Fitzgerald GA. In vivo indexes of platelet and vascular function during fish oil administration in patients with atherosclerosis. N Eng J Med 1986;314:937-942.
9. Olivieri O, Negri M, Gironcoli de M, et al. Effects of dietary fish oil on malondialdehyde production and gluthatione-peroxidase activity in hyperlipidemic patients. Scan J Clin Lab Invest 1988;48:659-665.

Reproduced with permission from Arthritis and Rheumatism

Chapter 4

NONSTEROIDAL ANTIINFLAMMATORY DRUG DEMAND DURING FISH OIL TREATMENT IN RHEUMATOID ARTHRITIS

Jacob E. Tulleken, Pieter C. Limburg, Wieke Wassenaar, Martin H. van Rijswijk.

SUMMARY

Dietary fish oil supplementation provides a beneficial effect on joint symptoms in rheumatoid arthritis (RA). Before we embark upon prescribing fish oil to large groups of patients emphasis should be given to determine its place in regular treatment for RA. Should it be used as adjunctive therapy or is it potent enough to replace more conventional treatment regimens? To answer this question we undertook a double blind, placebo controlled trial to investigate whether fish oil consumption could reduce indomethacin usage without deterioration of joint symptoms in 22 patients with RA. After a nine weeks run-in period the patients were randomly allocated to receive either fish oil (2.04 g 20:5,n-3 and 1.32 g 22:6,n-3) or coconut oil (control group) for twelve weeks. We report here a minor effect on the requirement of indomethacin in those patients who received fish oil (-19%, $p<0.05$), whereas no effect was observed in those patients who received placebo (-8%, NS). Between group comparison of percentages revealed no statistically significant differences in usage of indomethacin. No worsening of symptoms or alterations in the acute phase parameters were observed in both groups. We conclude that three months of fish oil supplementation could not significantly reduce indomethacin demand in RA patients.

INTRODUCTION

Dietary fish oil supplementation modifies the fatty acid composition of the membrane phospholipid fraction of platelets[1], erythrocytes[1], mononuclear cells[2], neutrophils[3] and others. The main long chain omega-3 polyunsaturated fatty acids (n-3 PUFA) in fish oil, eicosapentaenoic acid (EPA;20:5,n-3) and docosahexaenoic acid (DHA;22:6,n-3), competitively inhibit the formation of arachidonic acid (AA;20:4,n-6) derived metabolites of the cyclooxygenase and lipoxygenase pathway. Fish oil has considerable suppressing effects on thromboxane A2 (TXA2)[4], prostaglandin E2 (PGE2)[2] and leukotriene B4 (LTB4)[3] synthesis, while enhancing the production of weak, n-3 PUFA derived agonists like TXA3[4], PGE3[5] and LTB5[3]. This may result in anti-thrombotic and anti-inflammatory effects of fish oil as has been evaluated in cardiovascular diseases and in chronic inflammatory disorders like rheumatoid arthritis (RA). We and

others have demonstrated that daily fish oil consumption provides an additional suppressing effect on joint symptoms in RA patients continuing their regular treatment with non steroidal antiinflammatory drugs (NSAID) and 'disease modifying' antirheumatic drugs (DMARD)[3,6-10]. One of the mechanisms by which NSAID exert their effect is the inhibition of the production of prostaglandins whereas the production of leukotrienes remains unaffected. The ability of n-3 PUFA to affect both the cyclooxygenase and the lipoxygenase pathway should, theoretically, provide the possibility to reduce NSAID demand in RA patients. Therefore, we undertook a prospective double blind, placebo controlled trial to investigate whether fish oil consumption could reduce the need for indomethacin, as a model for other NSAID, in RA patients.

PATIENTS AND METHODS

Patients:

Twenty six patients who met the American Rheumatism Association criteria[11] for RA were asked to participate in the study. All fulfilled at least 3 of the following criteria for disease activity: duration of early morning stiffness>45 minutes, at least 6 tender joints, swelling in at least 3 joints and a C-reactive protein (CRP) level>6 mg/liter. Concurrent treatment for RA consisting of NSAID and DMARD was required to be stable for at least three months prior to the study. During the study the use of DMARD was kept constant. Informed consent was obtained from all patients.

Clinical protocol:

At the start of the trial regular NSAID treatment was discontinued and replaced by indomethacin suspension (5 mg/ml) and if necessary complemented with indomethacin suppository (25, 50 or 100 mg). The patients were required to discontinue any medication with analgesic capacities, other than indomethacin during the entire study. Furthermore they were encouraged to decrease their indomethacin usage without deterioration of joint symptoms. The daily usage of indomethacin was noted in a diary. Clinical evaluation was done every three weeks and consisted of: grip strength of each hand (kPa); Ritchie articular index[12]; the total number of swollen and tender joints; duration of early morning stiffness (in minutes); pain score on a visual analogue pain scale (VAS, 0-10, from no pain to worst ever). For the degree of joint pain (joint pain index) a scale of 0-3 was used (0=no tenderness, 1=mild, 2=moderate, 3=severe) and a scale of 0-2 (0=none, 1=moderate, 2=severe) was used to assess the degree of swelling of selected joints (joint swelling index).

Supplementation with fish oil and coconut oil:

After nine weeks (run-in period), the patients were randomly allocated on a numerically order of entry scheme by the pharmacy department of the hospital to enter either the fish oil or the control group for a 12 weeks treatment period. The patients in the fish oil group were treated with 12 fish oil capsules daily, primarily containing 20:5,n-3 (31 mol%) and 22:6,n-3 (22 mol%) fatty acids (ethyl esters), resulting in a daily supply of 2.04 g 20:5,n-3 and 1.32 g 22:6,n-3. The total daily dosage of fish oil for each patient was 6 g. Patients in the control group received the same amount of

identical appearing capsules with coconut oil primarily containing caprylic acid (63 mol%) and capric acid (36 mol%)[3]. To prevent identification of the coconut oil, fish flavour was added to the capsules. Both oil supplements were a gift from Aerofako, Amersfoort, The Netherlands. Compliance was monitored by pill counts. Patients were asked to adhere to their regular dietary habits. Each patient kept a daily food diary.

Laboratory tests:
Standard laboratory tests were performed every visit including a complete blood cell count, erythrocyte sedimentation rate (ESR), plasma C-reactive protein (CRP) and IgM-rheumatoid factor (IgM-Rf).

Data processing and statistical analysis:
The mean indomethacin requirement was calculated over a seven day period (three days before the day of visit, the day of visit and three days after the day of visit to the hospital). A standard paired analysis of data was conducted to study the change from baseline, using the Wilcoxon test. The Mann Whitney U test for unpaired data was used for comparison of groups at baseline and after treatment. P values less than 0.05 were considered significant.

RESULTS

Two patients in the fish oil group were withdrawn from the study because of non-compliance. In the control group one patient withdrew not tolerating the size and number of the capsules and one withdrew for personal reasons not related to her participation in the study. Both supplements caused short term (days), mild gastro-intestinal discomforts (nausea, ructus, diarrhea). At the end of the study data for 11 fish oil and 11 coconut oil supplemented patients were available for evaluation (Table 1).

Table 1 Patient characteristics at study entry.

	Fish oil treated	Control
Age, years		
Mean	55	54
Range	(44-63)	(43-66)
Males/females	1/10	3/8
Disease duration, years		
Mean	16	16
Range	(6-31)	(5-27)
Current medication		
Nonsteroidal antiinflammatory drugs	11	11
Gold	1	3
D-penicillamine	4	4
Antimalarial agents	4	2

The clinical and laboratory indices of disease activity of both groups before and after the supplementation protocol are presented in Table 2. ESR and plasma CRP levels

were significantly lower in the group who received fish oil supplements compared to the control group, as a result of the randomization protocol.

Table 2 Laboratory and clinical indices of disease activity of patients before and after study.

	Fish oil treated		Control	
	Before	After	Before	After
ESR (mm)**	17.0 (5.0-76.0)	24.0 (9.0-74.0)	56.0 (36.0-107)	54.0 (30.-98.0)
CRP (mg/liter)**	6.0 (6.0-94.0)	6.0 (6.0-56.0)	44.0 (11.0-70.)	30.0 (9.0-83.0)
IgM-Rf (IU/ml)	130.0 (35.0-650)	180.0 (28.0-900)	48.0 (9.0-550.)	55.0 (8.0-450.)
Joint pain index	23.0 (2.0-68.0)	21.0 (0.0-62.0)	22.0 (7.0-92.0)	17.0 (0.0-68.0)
RAI	13.0 (2.0-25.0)	15.0 (0.0-36.0)	18.0 (7.0-42.0)	14.0 (0.0-39.0)
Painful joints	12.0 (2.0-31.0)	11.0 (0.0-36.0)	13.0 (5.0-42.0)	13.0 (0.0-34.0)
Joint swelling index	2.0 (0.0-22.0)	4.0 (0.0-17.0)	4.0 (0.0-20.0)	3.0 (0.0-11.0)
Swollen joints	2.0 (0.0-20.0)	4.0 (0.0-16.0)	4.0 (0.0-18.0)	3.0 (0.0-11.0)
Grip strength (kPa)				
right hand	0.3.(0.1-0.8)	0.3 (0.1-1.1)	0.2 (0.0-0.4)	0.2 (0.0-0.5)
left hand	0.3 (0.1-0.8)	0.2 (0.1-1.1)	0.2 (0.0-1.2)	0.2 (0.0-1.4)
Stiffness (minutes)	60.0 (0.0-180)	30.0 (0.0-240)	30.0 (0.0-180)	15.0 (0.0-180)
VAS	2.5 (0.5-4.3)	2.3 (0.7-3.7)	2.7 (0.5-7.8)	1.3 (0.0-8.0)

* Values are the median (range). ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; IgM Rf = IgM rheumatoid factor; RAI = Ritchie articular index; VAS = visual analogue scale. ** $p<0.01$, for comparison of groups before treatment.

During the nine weeks run-in period the usage of indomethacin of all patients fell from: (median(range)) 70(18-150) mg/day to 56(25-157) mg/day but the difference was not significant. The influence of fish- and coconut oil supplementation on the individual usage of indomethacin in the following 12 weeks is shown in Figure 1. The patients were required to translate, if possible, any improvement of joint symptoms during fish oil or coconut oil treatment into a diminished indomethacin usage. The use of indomethacin fell to 81% (40-107) ($p<0.05$, from baseline) in the fish oil treated group and to 92% (0-130) (NS) in the control group. Between group comparison of percentages revealed no statistically significant differences (95% confidence limits for the difference between medians: -17 to 31, 2-alpha=0.62, beta=0.07).

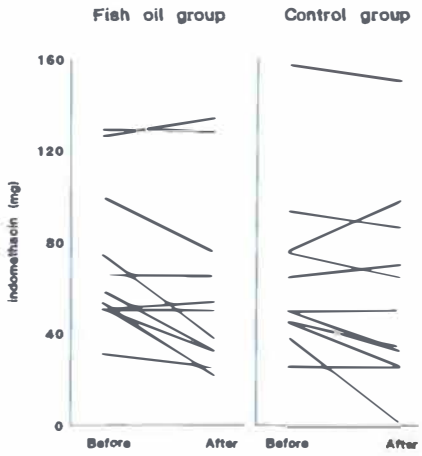


Figure 1 Mean daily indomethacin demand in the patients who received fish oil and in the patients who received coconut oil (control group). The use of indomethacin fell to 81% (40-107) ($p<0.05$) in the fish oil treated group and to 92% (0-130) (NS) in the control group.

DISCUSSION

The 20:4,n-6 derived prostaglandins and leukotrienes are potent local mediators of joint complaints and joint destruction in RA. Dietary n-3 PUFA, while increasing the 20:5,n-3 and 22:6,n-3 content of cell membrane phospholipids, competitively inhibit the formation of the proinflammatory metabolites PGE2 and LTB4. Recently, a 51% decrease of PGE2 release was measured in the supernatant of stimulated mononuclear cells of healthy volunteers who had received 4.6 g 20:5,n-3+22:6,n-3 daily for six weeks[2]. Previous investigators reported promising results of addition of fish oil or plant fatty acids to the diet in suppressing joint symptoms in RA patients. Belch et al[13] have shown that treatment with evening primrose oil, rich in gamma-linolenic acid (18:3,n-6), with or without 20:5,n-3 (240 mg) could allow some RA patients to reduce or even stop their NSAID. Evening primrose oil raises the di-homo-gamma-linolenic acid content in cell membrane phospholipids which is the precursor of PGE1, a prostaglandin with antiinflammatory properties. The combined therapy of the 18:3,n-6/20:5,n-3 group does not permit, however, a conclusion upon the effect of 20:5,n-3 on the required dosage of NSAID in RA.

We report here a minor effect on the requirement of indomethacin in those patients who received fish oil, whereas no effect was observed in those patients who received placebo. In contrast with our previous studies, using the same amount of dietary fish oil as a supplement to anti-rheumatic drugs, no significant alterations in clinical parameters of disease activity were observed[3,9]. This is not surprising since in the current study, patients were required to translate any improvement of joint symptoms into a diminished indomethacin usage. It is tempting to attribute the lack of a meaningful effect of 3.4 g/day of 20:5,n-3+22:6,n-3 for twelve weeks on indomethacin demand to the small sample size. However, the chance of a type II statistical error was approximately 7%, indicating the low impact of the sample size on the outcome of the study. Possibly, the fish oil-induced inhibition of the PGE2 production[2] is not enough to achieve similar clinical effects as indomethacin does. However, referring to the fact that indomethacin exerts some non prostaglandin dependent effects as well[14], it is possible that fish oil does not exert such effects and hence cannot replace indomethacin. Recently, it was reported that clinical improvement was more commonly observed after 24 weeks of treatment while using higher dosages of fish oil than we have used thus far[10]. The additional benefit was disappointing however, and it seems doubtful whether such a treatment schedule will lessen the need for NSAID. Especially when the high dosages of fish oil are provided in the semi-synthetic fatty acid ethyl ester (EE) form. Recent studies have assessed a marking diminished absorption of fish oil fatty acid EE, compared to fish oil in its natural, lower concentrated triglyceride form[15,16]. If so, this may raise doubts about the 'therapeutic' advantage of 'high' dose fish oil fatty acid EE. We emphasize the need for studies comparing the effects of fish oil EE and fish oil TG in RA.

The current available data provide little evidence that fish oil supplementation will lessen the need for indomethacin in RA patients. It is important to note however, that fish oil supplementation does possess potential antiinflammatory properties to affect RA disease activity. Among such effects are decreased LTB4 production and LTB4 mediated reactions such as chemotaxis and adhesion of both neutrophils[7] and

monocytes[17]. In addition, plasma levels of interleukin-1 correlate with ESR levels in RA patients[18] and fish oil supplementation has been shown to suppress the macrophage interleukin-1 production[27]. At present the exact meaning of these effects associated with fish oil supplementation is not clear, since in none of the trials it was reported that the ESR, rheumatoid factor titer, or the plasma levels of haemoglobin and CRP were altered. Large studies over more extended treatment periods are required to draw definitive conclusions on the ability of fish oil to favourably alter the progression and prognosis of the disease. Awaiting the results of such studies, we suggest that fish oil should not be considered as a substitute for nowadays NSAID and DMARD.

ACKNOWLEDGEMENTS

Dr. W.J. Sluiter is acknowledged for statistical advice.

REFERENCES

1. Schacky von C, Fisher S, Weber PC. Long-term effects of dietary marine n-3 fatty acids upon plasma and cellular lipids, platelet function and eicosanoid formation in humans. J Clin Invest 1985;76:1626-1631.
2. Endres S, Ghorbani R, Kelly VE, et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Eng J Med 1989;320:265-271.
3. Tempel vd H, Tulleken JE, Limburg PC, Muskiet FAJ, Rijswijk van MH. Effects of fish oil supplementation in rheumatoid arthritis. Ann Rheum Dis 1990;49:76-80.
4. Knapp HR, FitzGerald GA. The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. N Eng J Med 1989;320:1037-1043.
5. Gallon LS, Barcellu UO. Measurement of prostaglandin E3 and other eicosanoids in biologic samples using high pressure liquid chromatography and radioimmunoassay. Prostaglandins 1986;31:217-225.
6. Kremer JM, Jubiz W, Michalek AV, et al. Fish oil fatty acid supplementation in active rheumatoid arthritis. Ann Intern Med 1987;106:497-503.
7. Sperling I, Weinblatt M, Robin JL, et al. Effects of dietary supplementation with marine fish oil on leukocyte lipid mediator generation and function in rheumatoid arthritis. Arthritis Rheum 1987;30:988-997.
8. Cleland LG, French JK, Betts WH, Murphy GA, Elliot MJ. Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. J Rheumatol 1988;15:1471-1475.
9. Tulleken JE, Limburg PC, Muskiet FAJ, van Rijswijk MH. Vitamin E status during dietary fish oil supplementation in rheumatoid arthritis. Arthritis Rheum, 1990;33:1416-1419.
10. Kremer JM, Lawrence DA, Jubiz W, et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Arthritis Rheum 1990;33:810-820.
11. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheuamtism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-324.
12. Ritchie DM, Boyle JA, McInnes JM, et al. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. Quart J Med 1968;37:393-406.
13. Belch JJF, Ansell D, Madhok R, O'Dowdd A, Sturrock RD. Effects of altering dietary essential fatty acids on requirements for non-steroidal anti-inflammatory drugs in patients with rheumatoid arthritis: a double blind placebo controlled study. Ann Rheum Dis 1988;47:96-104.
14. Abramson SB, Weissmann G. The mechanisms of action of non steroidal antiinflammatory drugs. Arthritis Rheum 1989;1:1-9.
15. Lawson D, Hughes BG. Human absorption of fish oil fatty acids as triacylglycerols, free fatty acids, or ethyl esters. Biochem Biophys Res Comm 1988;152:328-335.

16. Lawson LD, Hughes BG. Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethylesters co-ingested with a high fat meal. Biochem Biophys Res Commun 1988;156:960-963.
17. Schmidt EB, Nielsen LK, Pedersen JO, Kornerup HJ, Dyerberg J. The effect of n-3 poly-unsaturated fatty acids on lipids, platelet function, coagulation, fibrinolysis and monocyte chemo-taxis in patients with hypertension. Clin Chim Acta 1990;189:25-32.
18. Eastgate JA, Wood NC, Di Giovine FS, Symons JA, Grinlinton FM, Duff GW. Correlation of plasma interleukin 1 levels with disease activity in rheumatoid arthritis. Lancet, 1988;I:706-709.

Chapter 5

A COMPARISON BETWEEN THE EFFECTS OF FISH OIL FATTY ACID ETHYL ESTERS AND FISH OIL TRIGLYCERIDES IN RHEUMATOID ARTHRITIS

Jacob E. Tulleken, Pieter C. Limburg, Frits A.J. Muskiet,
Karin M. Kazemier, Martin H. van Rijswijk.

SUMMARY

Using a twelve weeks, double blind, study design we compared the efficacy of 'high' dose fish oil fatty acid ethyl ester (EE) and 'low' dose fish oil triglyceride (TG) preparations for altering fatty acid compositions of plasma cholesterol esters and neutrophil phospholipids, neutrophil leukotriene production and disease activity in 29 patients with rheumatoid arthritis. The 'high' dose fish oil fatty acid EE preparation resulted in a daily supply of 2.04 g eicosapentaenoic acid (20:5,n-3) and 1.32 g docosahexaenoic acid (22:6,n-3). 'Low' dose TG supplementation resulted in a daily supply of 1.08 g 20:5,n-3 and 0.72 g 22:6,n-3. Despite the difference in the actual amount of 20:5,n-3 and 22:6,n-3 present in the capsules, both fish oil preparations were equally effective in incorporation of 20:5,n-3 in plasma cholesterol esters and neutrophil phospholipids, inhibition of in vitro neutrophil leukotriene B4 production and induction of leukotriene B5 formation, and in suppressing joint symptoms. The relative amount of arachidonic acid in plasma cholesterol esters and neutrophil phospholipids was minimally changed by both fish oil preparations. This study reveals that, apart from the actual amount of fish oil fatty acids present in the capsules, the chemical form in which they are provided (EE or TG) is of importance to the induced clinical and laboratory effects in rheumatoid arthritis.

INTRODUCTION

Dietary fish oil supplementation has been used as a treatment modality to suppress inflammation in patients with rheumatoid arthritis (RA)[1,2,3,4]. The main long chain omega-3 polyunsaturated fatty acids (n-3 PUFA) in fish oil, eicosapentaenoic (20:5,n-3) and docosahexaenoic (22:6,n-3) acids competitively inhibit the formation of the cyclo-oxygenase and lipoxygenase metabolites of arachidonic acid (20:4,n-6). Fish oil has considerable suppressive effects on prostaglandin E2 (PGE2)[5] and leukotriene B4 (LTB4)[1,2,6] synthesis, and enhances the production of less active, n-3 PUFA derived, metabolites like PGE3[7] and LTB5[1,2,6]. It is assumed that the relief of joint symptoms in RA patients during fish oil supplementation is accounted for by altered production of prostaglandins and leukotrienes[1,2]. In a placebo controlled trial, Kremer et al. were the first to demonstrate clinical improvements with a daily dose of 4.5 g n-3 PUFA[1]. We have shown similar effects at supplementation of 3.4 g n-3 PUFA/day[2,3]. Recently it was demonstrated that improvement of joint symptoms was more common when RA patients

were supplemented with 90 mg n-3 PUFA/kg/day compared with supplementation of 45 mg n-3 PUFA/kg/day[21]. The additional clinical benefit of the higher dosage was disappointing however, whereas there was no significant difference between the two dosages to affect mononuclear LTB4 synthesis. This suggests that both dosages altered the fatty acid composition of the mononuclear membrane phospholipid fraction to the same extent. As this fraction reflects precursor availability for leukotriene synthesis, the findings raise doubts about the advantage of 'high' dose fish oil supplementation. The fish oil fatty acids in the studies discussed above were administered in the semi-synthetic ethyl ester (EE) form, which allows much higher concentrations of n-3 PUFA compared to the relative amounts in the naturally occurring fish oil triglycerides (TG). Whether the clinical and laboratory effects are dependent on the chemical form of n-3 PUFA has not been well characterized yet. Recent studies have assessed a diminished absorption of fish oil EE- compared to fish oil TG preparations[8,9]. If so, this may raise doubts about the therapeutic advantage of 'high' dose fish oil fatty acid EE over 'low' dose fish oil preparations in their natural TG form. In the present double blind study we compared the efficacies of 'high' dose fatty acid EE- and 'low' dose TG fish oil preparations in altering the fatty acid compositions of plasma CE and neutrophil PL, leukotriene production, and disease activity in patients with RA.

PATIENTS AND METHODS

Patients and study design:

Twenty-nine patients with RA (Table 1), as defined by the American Rheumatism Association[10] criteria, participated in the study. Prior treatment was required to be stable for at least three months and continued to be so during the study. Patients were asked to adhere to their usual dietary habits and to keep a food diary. Informed consent was obtained. To ensure that the patients had stable disease

Table 1 Baseline characteristics and dietary compositions of the patients randomly assigned to receive either 'high' dose fish oil fatty acid EE or 'low' dose fish oil TG supplementation.

	'HIGH' DOSE EE	'LOW' DOSE TG
Age mean(range)*	60 (37-75)	59 (31-80)
Sex (Male:Female)	5:10	4:10
Duration of disease* mean(range)	16 (5-39)	22 (5-28)
Current Medication**		
NSAID	10	11
Gold	1	3
d-Penicillamine	5	6
Anti-malarials	1	1
None	4	2
Composition of diets ***		
Energy intake (kcal/day)	1790 (378)	1889 (485)
Fat (energy %)	36 (8)	40 (5)
Carbohydrates (energy %)	45 (10)	44 (7)
Proteins (energy %)	16 (4)	14 (3)
P/S ratio	0.5 (0.3)	0.5 (0.2)

* years; ** number of patients; *** values are presented as mean ±SD. Compositions of the diets were calculated from 3-day food diaries recorded by the patients; differences were not statistically significant between groups.

activity a six weeks run-in period was used for premonitoring relevant clinical signs and symptoms. At week six the patients were randomly assigned to receive either 'high' or 'low' dose of fish oil for twelve weeks, followed by a six weeks wash out period. The code of randomization was maintained in the pharmacy department of the hospital.

Supplementation of the fish oil preparations:
The total fatty acid compositions of the supplements are given in Table 2.

Table 2 Fatty acid compositions of the fish oil preparations (mol/100 mol).

Fatty acids	'LOW' DOSE TG	'HIGH' DOSE EE
SAFA	30.86	7.68
10:0	0.15	0.12
12:0	0.39	0.02
14:0	8.74	0.49
16:0	18.32	2.65
18:0	2.88	3.86
20:0	0.16	0.38
22:0	0.10	0.13
24:0	0.10	-
MUFA	26.71	24.98
16:1,n-7	9.53	0.81
18:1,n-7	3.90	4.26
20:1,n-7	0.20	0.50
18:1,n-9	9.08	9.40
20:1,n-9	1.59	4.34
22:1,n-9	0.32	0.68
24:1,n-9	0.59	0.51
22:1,n-11	0.32	4.48
PUFA	42.43	67.34
18:3,n-3	0.77	1.02
18:4,n-3	3.20	3.37
20:4,n-3	0.85	1.99
20:5,n-3	18.62	31.68
22:5,n-3	2.39	3.80
22:6,n-3	12.76	21.51
18:2,n-6	1.32	1.51
18:3,n-6	0.22	0.16
20:2,n-6	0.19	0.45
20:3,n-6	0.19	0.31
20:4,n-6	1.44	0.99
22:4,n-6	0.18	0.16
22:5,n-6	0.30	0.39
n-3	38.59	63.37
n-6	3.84	3.97

The fish oil supplements were given daily as 12 identically appearing capsules containing 500 mg oil/capsule for a total duration of three months. Both supplements were generously provided by Aerofako, The Netherlands. The 'high' dose fish oil fatty acid EE preparation resulted in a daily supply of 2.04 g 20:5,n-3 and 1.32 g 22:6,n-3 and a total of 6 g oil. The 'low' dose TG preparation contained about half the amount of fish oil fat as present in the 'high' dose capsules. This resulted in a daily supply of 1.08 g 20:5,n-3 and 0.72 g 22:6,n-3 and a total of 6 g oil. The alpha-tocopherol content of both types of fish oil capsules was 5 micromol/ml oil.

Clinical evaluation:
Clinical evaluation was done every other week. It consisted of the documentation of duration of early morning stiffness (in minutes), Ritchie articular index[11], joint pain index (on a four point scale: 0=absent, 1=mild, 2=moderate, 3=severe), joint swelling index (on a three point scale: 0=absent, 1=moderate, 2=severe), and visual analogue pain scale (VAS; on a ten point scale from 0 = no pain to 10 = worst ever)). Grip strength was measured using a manometer (kPa).

Laboratory evaluation:
Laboratory evaluation of peripheral blood parameters was performed every two weeks. It comprised determinations of a complete blood cell count (Coulter counter), erythrocyte sedimentation rate (ESR), and the assays of the plasma levels of IgM-rheumatoid factor (IgM-Rf)[12], C-reactive protein (CRP)[13] and fibrinogen[14].

Assessment of in vitro production of leukotrienes:
At the ends of the run-in, supplementation and wash out periods neutrophils were isolated from 30 ml of peripheral blood and resuspended to a final concentration of 10^7 cells/ml. After 10 min. stimulation with 10 umol/l calcium-ionophore A23187 the released leukotrienes were measured by high performance liquid chromatography using a C18 column[2,15].

Determination of oil supplement fatty acid composition:
Analyses of both medium and long chain fatty acids in the fish oil capsules were performed by a capillary gas chromatographic quantification technique, as described previously[16].

Determination of plasma CE fatty acid composition:
After preparation of a plasma total lipid extract and isolation of the CE fraction by amino propyl-silica columns, its fatty acid composition was determined by capillary gas chromatography[17].

Determination of neutrophil PL fatty acid composition:
At the ends of the run-in and supplementation periods lipids from neutrophils were extracted with chloroform/methanol. The total PL fraction was isolated by high performance liquid chromatography, essentially as described previously[18,19]. Its fatty acid composition was determined by capillary gas chromatography with flame ionization detection using an apolar stationary phase[20].

Data processing and statistical analyses:
The values are presented as median(range) unless otherwise indicated. A standard analysis of data was conducted to study the change from baseline, using the Wilcoxon test. Comparison of changes from baseline between groups was assessed using the Mann-Whitney U test for unpaired data. The p value for each of the response variables was considered significant if less than 0.05. The Spearman correlation coefficient was calculated to determine relationships between variables.

RESULTS

Thirty two patients entered the trial at the beginning of the six weeks run-in period. At the end of this period three patients were excluded from further study because of increased clinical disease activity, necessitating a change of 'disease modifying anti-rheumatic drugs'. The food diary revealed no differences in the macronutrient compositions of the diet between groups (see Table 1). Both fish oil supplements initially induced apparently similar, short term, gastro-intestinal discomforts (nausea, ructus and diarrhea). All participants endured the fish oil supplementation without any specific problems.

Clinical evaluation:

Table 3 shows the effects of the two fish oil preparations on clinical disease parameters,

Table 3 The effect of two fish oil dosages on clinical and laboratory disease parameters, before, after twelve weeks and the following six weeks wash-out period in patients with rheumatoid arthritis.

		'LOW' DOSE TG	'HIGH' DOSE EE
JPI	before	17.0 (1.0-85.0)	19.0 (0.0-122.0)
	after	4.0 (0.0-84.0)*	11.0 (2.0-70.0)**
	wash-out	8.0 (0.0-66.0)	12.0 (1.0-78.0)*
JSI	before	3.0 (0.0-15.0)	5.0 (0.0-28.0)
	after	3.0 (0.0-12.0)	2.0 (0.0-14.0)***
	wash-out	5.0 (0.0-8.0)	6.0 (0.0-16.0)
Grip str. R (kPa)	before	0.3 (0.0-0.8)	0.2 (0.0-1.2)
	after	0.3 (0.0-0.9)	0.2 (0.0-1.2)
	wash out	0.3 (0.0-0.9)	0.3 (0.0-1.2)
Grip str. L (kPa)	before	0.3 (0.0-0.9)	0.2 (0.0-1.1)
	after	0.4 (0.0-1.2)	0.2 (0.0-1.0)
	wash-out	0.3 (0.0-1.2)	0.2 (0-1.2)
Stiffness (min)	before	17.0 (0.0-120.0)	45.0 (0.0-120.0)
	after	0.0 (0.0-120.0)	15.0 (0.0-60.0)***
	wash-out	0.0 (0.0-120.0)	15.0 (0.0-60.0)***
Ritchie index	before	11.0 (1.0-35.0)	9.0 (0.0-60.0)
	after	4.0 (0.0-39.0)**	8.0 (2.0-48.0)
	wash-out	7.5 (0.0-31.0)***	9.0 (1.0-41.0)
VAS	before	4.5 (0.0-9.0)	4.5 (0.8-8.0)
	after	2.8 (0.0-9.0)***	3.0 (0.5-8.0)
	wash-out	4.4 (0-7.9)	4.0 (0.8-6.9)
ESR (mm)	before	41.0 (2.0-84.0)	31.0 (14.0-90.0)
	after	40.0 (4.0-80.0)	35.0 (8.0-105.0)
	wash-out	42.0 (2.0-80.0)	35.0 (9.0-102.0)
CRP (mg/l)	before	10.0 (6.0-55.0)	13.0 (6.0-56.0)
	after	11.0 (6.0-34.0)	16.0 (6.0-65.0)
	wash-out	6.0 (6.0-64.0)	23.0 (6.0-71.0)
Fibrinogen (g/l)	before	3.9 (2.2-5.0)	4.3 (2.3-4.8)
	after	3.5 (1.8-4.4)***	4.0 (2.2-5.4)
	wash-out	3.4 (1.6-4.6)**	4.1 (2.2-5.2)
IgM-Rf (IU/ml)	before	60.0 (3.0-850.0)	48.0 (3.0-900.0)
	after	55.0 (3.0-400.0)	50.0 (3.0-1000.0)
	wash-out	40.0 (3.0-900.0)*	40.0 (3.0-900.0)
Haemoglobin (g/l)	before	129.0 (91.0-172.0)	127.0 (95.0-160.0)
	after	128.0 (101.0-170.0)	133.0 (90.0-151.0)**
	wash-out	128.0 (101.0-174.0)	130.0 (92.0-145.0)***
Platelets (10^9/l)	before	294.0 (157.0-568.0)	259.0 (198.0-458.0)
	after	254.0 (139.0-517.0)*	264.0 (166.0-470.0)
	wash-out	283.0 (154.0-521.0)	282.0 (172.0-437.0)

* $p < 0.01$; ** $p < 0.03$; *** $p < 0.05$ significant from the level before dietary n-3 supplementation. JPI = Joint pain index; JSI = Joint swelling index; Grip str. = Grip strength, R = right hand, L = left hand.

as established at the ends of the run-in, supplementation and wash out periods. When treatment endpoints were compared with baseline values, both 'high' dose EE and 'low' dose TG fish oil supplementations resulted in clinical improvements. The difference between absolute changes (week 18-week 6) in clinical disease variables between the two fish oil preparations, did not achieve statistical significance. Six weeks after discontinuation of fish oil supplementation comparison of clinical parameters with the end of the run-in period values revealed significant improvements in joint pain index and duration of early morning stiffness in the 'high' dose fish oil fatty acid EE group. In the 'low' dose fish oil TG group the decrease of the Ritchie articular index persisted in the six weeks wash-out period.

Laboratory evaluation:
The effect of the two fish oil dosages on laboratory parameters at the ends of the run-in, supplementation and wash out periods are also shown in Table 3. 'Low' dose fish oil TG supplementation resulted in a moderate decrease in plasma fibrinogen levels ($p<0.05$) that persisted in the wash out period, and in a fall in the median number of platelets from 294 x 10^9/l at baseline to 254 x 10^9/l ($p<0.02$). 'High' dose fish oil fatty acid EE supplementation led to a slight increase in serum haemoglobin levels ($p<0.03$).

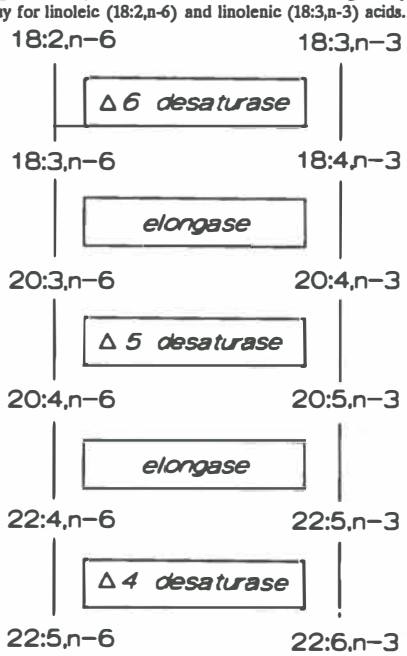
Neutrophil membrane fatty acid composition:
Table 4 depicts the fatty acid composition of the total PL fractions of isolated neutrophils at the ends of the run-in and supplementation periods of both fish oil supplements. Owing to technical problems, the fatty acid composition of neutrophil membrane PL was measured for 11 out of 15 patients in the 'high' dose fish oil EE group and 13 out of 14 patients in the 'low' dose fish oil TG group. The remaining patients of both groups were, however, comparable in terms of demographic data, use of medications and dietary habits. Except for 24:1,n-9 in the 'high' dose group neither saturated (SAFA) nor monounsaturated fatty acids (MUFA) were affected by either of the fish oil supplements. Only the 'high' dose fish oil fatty acid EE led to a significant increase of 22:6,n-3 ($p<0.01$), whereas both supplements increased the relative amounts of 20:5,n-3 ($p<0.01$) and 22:5,n-3 ($p<0.01$). Neutrophil PL of the 'high' dose fish oil fatty acid EE group did not incorporate significantly more 20:5,n-3 than those in the 'low' dose fish oil TG group. In both groups, incorporation of the n-3 PUFA notably occurred at the expense of the n-6 PUFA, like 20:3,n-6 and 22:4,n-6. The relative amount of 20:4,n-6 was minimally decreased in the 'low' dose group only ($p<0.01$). Comparison of absolute or percentage treatment effects revealed no significant difference in the efficacies of both supplements to alter the fatty acid composition of neutrophil PL.

Table 4 Fatty acid composition of the neutrophil total phospholipid fraction before and after twelve weeks of supplementation of two different fish oil preparations in patients with rheumatoid arthritis. The fatty acids are presented as mol/100 mol.

	'LOW' DOSE TG		'HIGH' DOSE EE	
	before	after	before	after
SAFA	57.9 (51.3-75.9)	57.8 (49.0-73.7)	58.6 (51.9-72.3)	61.2 (51.6-67.2)
14:0	1.8 (0.3-3.3)	1.8 (1.2-3.3)	1.8 (1.2-3.2)	1.7 (1.3- 2.9)
16:0	34.3 (22.5-47.9)	34.1 (27.4-41.9)	34.9 (31.0-47.1)	36.0 (31.0-44.5)
18:0	20.7 (17.7-32.2)	22.2 (17.3-31.4)	20.3 (17.3-25.6)	20.4 (17.5-24.6)
24:0	1.3 (0.8-1.7)	1.2 (0.9-1.7)	1.3 (0.7-1.9)	1.3 (0.8-1.6)
MUFA	27.9 (13.3-32.0)	28.0 (15.9-33.5)	25.7 (16.4-33.5)	22.6 (19.7-34.0)
18:1,n-7	2.4 (1.0-3.0)	2.4 (1.1-4.0)	1.9 (1.4-2.8)	1.7 (1.4-2.7)
18:1,n-9	22.6 (11.1-26.2)	22.9 (13.1-27.7)	21.7 (13.2-28.3)	19.1 (16.2-28.8)
24:1,n-9	2.6 (1.2-4.2)	2.8 (1.6-3.2)	2.6 (1.5-4.8)	2.1 (1.7-3.1)***
PUFA	15.0 (10.7-17.1)	13.3 (10.4-22.3)	14.7 (8.4-19.0)	14.4 (10.1-18.3)
18:3,n-3	0.3 (0.28-0.5)	0.3 (0.1-0.5)	0.3 (0.2-0.6)	0.3 (0.2-0.4)
20:5,n-3	0.1 (0.0-0.3)	0.6 (0.4-1.4)*	0.1 (0.1- 0.3)	0.8 (0.4-1.3)*
22:5,n-3	0.4 (0.2-0.8)	0.8 (0.5-1.3)*	0.5 (0.2-0.8)	0.7 (0.2-1.0)*
22:6,n-3	0.6 (0.3-1.8)	0.6 (0.3-1.5)	0.6 (0.2-1.6)	0.7 (0.4-1.6)*
18:2,n-6	7.3 (3.6-10.6)	6.8 (3.9-11.0)	6.9 (4.0-10.2)	7.4 (3.6-10.1)
20:3,n-6	0.8 (0.6-1.1)	0.6 (0.3-0.7)*	0.7 (0.5-0.9)	0.5 (0.4- 0.7)*
20:4,n-6	4.2 (3.1-6.4)	3.5 (2.1-6.2)*	3.9 (2.7-5.5)	3.9 (2.9- 5.6)
22:4,n-6	0.8 (0.5-1.3)	0.4 (0.2-0.6)*	0.7 (0.5-1.2)	0.4 (0.2-0.7)*
PUFA/SAFA	0.3 (0.1-0.3)	0.2 (0.1-0.5)	0.3 (0.1-0.4)	0.3 (0.2-0.3)
n-3	1.5 (0.9-2.3)	2.4 (1.8-3.8)*	1.4 (0.7-2.9)	2.7 (1.4-4.1)*
n-6	13.2 (8.4-15.8)	11.0 (8.2-18.5)**	12.9 (7.7-17.1)	12.4 (7.3-15.5)
n-7	2.4 (1.0-3.0)	2.4 (1.1-4.0)	1.9 (1.4-2.8)	1.7 (1.4-2.7)
n-9	25.4 (12.2-29.3)	25.5 (14.8-30.5)	24.4 (14.8-31.4)	21.2 (18.1-31.9)
DBI	0.7 (0.5-0.8)	0.7 (0.5-1.0)	0.7 (0.5-0.8)	0.7 (0.6-0.8)

* $p < 0.01$; ** $p < 0.02$; *** $p < 0.05$, significant from the level before supplementation. DBI=double bond index.

Figure 1 Fatty acid desaturation and chain elongation pathway for linoleic (18:2,n-6) and linolenic (18:3,n-3) acids.



Median values of some fatty acid product/precursor ratios, relating to desaturase and chain elongation activities (see Figure 1) in neutrophil PL at the ends of the run-in and supplementation periods of both fish oil preparations, are shown in Table 5.

Table 5 Median values for fatty acid product/precursor ratios in neutrophil phospholipids of patients with RA before and at the end of supplementation of 'low' dose TG or 'high' dose fish oil fatty acid EE.

	Before	After
Ratios	'LOW' DOSE TG	
20:3,n-6/18:2,n-6	0.10	0.09*
20:4,n-6/20:3,n-6	5.71	5.82
22:4,n-6/20:4,n-6	0.19	0.12*
	'HIGH' DOSE EE	
20:3,n-6/18:2,n-6	0.11	0.07*
20:4,n-6/20:3,n-6	5.95	7.07*
22:4,n-6/20:4,n-6	0.17	0.09*

* $p<0.01$, versus presupplementation values

In vitro leukotriene production:

The effects of supplementation of the two fish oil preparations on the production of LTB4 and LTB5 by neutrophils are shown in Table 6. Comparison of treatment effects of both dosages showed a slightly more pronounced suppression of LTB4 production ($p<0.01$, 90% confidence limits= 11 to 47, for the difference between medians) during 'low' dose fish oil TG supplementation then during 'high' dose fish oil fatty acid EE supplementation. In 5 patients LTB5 production was detectable before the start of the oil supplements. Comparison of treatment effects revealed no significant differences in the efficacies of both dosages to increase LTB5 production. Six weeks after discontinuation of fish oil supplementation both LTB4 and LTB5 production had restored to the levels at the end of the run-in period in most patients. The changes in clinical disease variables did not correlate with changes in leukotriene production.

Table 6 The effects of the two fish oil preparations at the ends of the run-in, supplementation and wash out periods on in vitro leukotriene production (ng/10^7 cells, 10 min) by neutrophils isolated from the blood of patients with rheumatoid arthritis.

Variables	Before	After	Wash-out
		'LOW' DOSE TG	
Leukotriene B4	180.0 (155.0-237.0)	146.0 (94.0-204.0)*	180.0 (116.0-231.0)
Leukotriene B5	0.0 (0.0-6.0)	17.0 (9.0-24.0)*	2.0 (0.0-11.0)**
		'HIGH' DOSE EE	
Leukotriene B4	186.0 (129.0-241.0)	160.0 (132.0-246.0)*	175.0 (140.0-234.0)
Leukotriene B5	0.0 (0.0-4.0)	19.0 (9.0-28.0)*	4.0 (0.0-10.0)**

* $p<0.01$, ** $p<0.05$ versus presupplementation values.

Plasma CE fatty acid composition:

Table 7 depicts the fatty acid composition of the plasma CE fraction at the ends of the run-in and supplementation periods of either 'high' dose fish oil fatty acid EE and 'low'

Table 7 Selected fatty acids (mol/100 mol) in the plasma CE fraction during supplementation with two different fish oil preparations.

	'LOW' DOSE TG		'HIGH' DOSE EE	
	Before	After	Before	After
SAFA	16.8 (13.8-22.6)	17.5 (13.7-21.6)	17.1 (13.9-20.1)	18.9 (14.5-22.7)*
14:0	1.2 (0.6-1.6)	1.2 (0.5-2.0)	1.2 (0.6-1.8)	1.3 (0.6-2.0)
16:0	13.8 (2.0-19.9)	15.0 (12.0-18.4)**	14.0 (12.0-16.7)	15.4 (12.8-19.7)*
18:0	1.6 (1.0-2.1)	1.5 (1.1-1.9)	1.5 (1.0-2.6)	1.7 (1.1-2.0)
MUFA	21.7 (15.9-31.8)	21.6 (17.2-30.9)	21.2 (16.6-31.3)	22.1 (16.7-28.8)
16:1,n-7	2.8 (0.8-9.0)	2.7 (1.5-7.1)	3.2 (2.4-5.9)	2.7 (2.0-4.8)**
18:1,n-7	1.3 (1.1-1.4)	1.5 (1.2-1.7)*	1.3 (1.1-1.7)	1.4 (1.2-2.2)**
18:1,n-9	17.4 (13.2-21.7)	17.4 (13.8-22.6)	16.1 (12.3-24.0)	17.5 (13.0-21.9)
PUFA	60.8 (52.3-70.3)	60.7 (51.6-67.7)	61.4 (48.7-67.7)	59.1 (48.8-67.0)
18:3,n-3	0.5 (0.1-1.3)	0.6 (0.4-1.4)	0.6 (0.4-0.9)	0.6 (0.3-1.1)
20:5,n-3	0.5 (0.3-2.0)	4.0 (3.0-6.2)*	0.5 (0.3-1.7)	4.4 (2.6-8.2)*
22:6,n-3	0.4 (0.1-1.1)	0.7 (0.1-1.5)**	0.4 (0.2-0.7)	0.8 (0.2-1.4)*
18:2,n-6	52.7 (44.5-61.9)	47.9 (41.7-56.6)*	52.8 (41.5-59.5)	47.0 (37.4-57.1)*
18:3,n-6	0.5 (0.3-1.1)	0.3 (0.2-0.9)*	0.6 (0.4-1.2)	0.4 (0.2-0.8)*
20:3,n-6	0.6 (0.5-0.9)	0.5 (0.3-0.7)	0.7 (0.4-1.4)	0.5 (0.2-0.7)
20:4,n-6	5.6 (3.6-6.7)	5.2 (4.0-6.7)	5.2 (3.6-7.3)	5.4 (3.9-7.1)
PUFA/SAFA	3.5 (2.4-5.1)	3.5 (2.6-4.9)**	3.5 (2.4-4.9)	3.1 (2.2-4.6)*
n-3	1.5 (1.0-3.2)	5.3 (4.0-8.0)*	1.5 (1.0-3.3)	5.7 (3.7-10.3)*
n-6	59.2 (50.3-68.9)	54.2 (46.8-62.3)*	59.8 (47.2-66.0)	52.9 (43.6-63.1)*
n-7	4.2 (2.0-10.1)	4.2 (2.7-8.3)	4.4 (3.6-7.2)	4.1 (3.6-6.8)
DBI	1.6 (1.5-1.7)	1.7 (1.5-1.8)*	1.6 (1.4-1.7)	1.7 (1.5-1.9)*

* $p < 0.01$; ** $p < 0.05$; significantly different from the level before n-3 supplementation.

dose fish oil TG. Changes in relative amounts of n-3 and n-6 PUFA in the plasma CE were more pronounced than those observed in the neutrophil membrane PL. Relative amounts of 20:4,n-6 were not significantly altered by either of the fish oil preparations. In agreement with the findings in the neutrophil PL both dosages were equally effective in increasing the n-3 PUFA content in the plasma CE fraction.

DISCUSSION

This trial confirms previous observations that dietary fish oil supplementation is effective in suppressing joint symptoms in RA patients[1,2,3,4]. From a clinical point of view, the lack of a concurrent control group makes interpretation of the induced changes in joint symptoms difficult. However, in earlier, placebo controlled, studies the beneficial clinical effects of the currently used 'high' dose fish oil EE were significantly greater than the ones achieved with placebo[2,3]. Comparison of the clinical data with those obtained from an historical control group confirmed the benefit of both fish oil preparations over placebo[2,3] (data not shown). Whether the clinical and laboratory effects are dependent on the chemical form of n-3 PUFA has not been well characterized. Several studies have shown the therapeutic effectiveness of supplementation of fish oil fatty acids in the EE form[2,3]. We report here that a TG fish oil preparation, while containing half the amount of n-3 PUFA/capsule, is almost equally effective in changing clinical disease parameters as a 'high' dose fish oil EE preparation. The lack of a dose response relationship on clinical disease variables between the two fish oil preparations in the current study is not surprising in the light of the induced changes in the fatty acid compositions of neutrophil PL and plasma CE. The neutrophil PL and plasma CE fractions of the 'high' dose fish oil fatty acid EE group did not incorporate more 20:5,n-3 than the ones in the 'low' dose TG group. Considering the incorporation of n-3 PUFA into plasma CE as an indicator of gastro-intestinal absorption, our findings suggest a diminished bioavailability of fish oil fatty acid EE when compared with fish oil TG preparations. This is in agreement with several trials that showed diminished absorption of 20:5,n-3 and 22:6,n-3 in EE form compared with those administered in TG form[8,9,22]. In contrast, others have demonstrated that fish oil TG and EE preparations that contained equal amounts of n-3 PUFA enriched plasma lipids with 20:5,n-3 to the same extent[23]. Our findings raise doubts on the extra profit of highly concentrated fish oil EE preparations in comparison with lower concentrated fish oil preparations in the natural TG form. It is not clear to what extent the apparently diminished bioavailabilty of the EE is influenced by the daily "background" diet. Recently, it was reported that the absorption of fish oil EE is dependent upon the amount of co-ingested fat[8]. In addition, once absorbed, the n-6 PUFA and n-3 PUFA compete for the same binding site on the sn-2 position of the glycerol moiety in PL[24]. In this context the dietary intake of linoleic acid (18:2,n-6) may affect the incorporation of n-3 PUFA in cel membranes and thereby its efficacy to alter eicosanoid synthesis. In the present study all patients completed a food diary. It appeared, however, to be an insensitive method to detect delicate differences in dietary fat intake between the two treatment groups, and could not be used to explain the observed membrane PL and plasma CE fatty acid profiles.

Like in plasma CE, the incorporation of n-3 PUFA in neutrophil PL occurred notably at the expense of n-6 PUFA, although the relative amounts of 20:4,n-6 were minimal-

ly changed. Similar results were obtained by others[25-29] who showed that daily administration of 1 to 6 g fish oil did not seriously alter the relative amounts of 20:4,n-6 in neutrophils, erythrocytes or platelets. However, not all studies are consistent[6]. In experimental models, it has been established that there is marked retention of 20:4,n-6 in certain phospholipids during essential fatty acid deficiency[30]. The 20:4,n-6 in neutrophil PL originates principally from microsomal desaturation and elongation of dietary 18:2,n-6 (Figure 1). Dietary long chain n-3 PUFA interfere with n-6 PUFA metabolism by reducing delta-6 desaturase activity, thus affecting the rate limiting step in the conversion of 18:2,n-6 to 20:4,n-6[31]. Analogously, the decreased 20:3,n-6/18:2,n-6 ratio in the neutrophil PL fractions noticed in the present study suggests a diminished delta-6 desaturase and/or chain elongation activity. The fact that, despite diminished conversion of 18:2,n-6, the relative concentrations of 20:4,n-6 remained stable may partially be explained by increased retroconversion of 22:4,n-6 to 20:4,n-6. Like 22:6,n-3 for 20:5,n-3[32], the 22:4,n-6 appears to be a reservoir for 20:4,n-6[33]. We do realize that the alleged changes in enzyme activities are based upon product/precursor ratios and are therefore highly speculative. Further studies in which enzyme activities are measured to elucidate essential fatty acid modification in tissues during dietary fish oil supplementation are warranted.

The changes in fatty acid composition of neutrophil PL induced by both fish oil preparations resulted in a moderate decrease in LTB4 production, concomitant with the generation of small amounts of 20:5,n-3 derived LTB5. As shown before[2], but in contrast to others[1] no correlations were found between changes in the in vitro leukotriene production and changes in clinical disease variables. Possibly, this is to be explained by the unphysiological way of stimulation of neutrophils to produce leukotrienes and the difficulty to assess the degree of patient's joint pain and swelling. Nevertheless, it is generally assumed that relief of joint symptoms in RA patients during fish oil supplementation is accounted for by altered production of leukotrienes and prostaglandins and mediated reactions. As yet no information is available on the optimum dose of fish oil required to modulate eicosanoid synthesis. In the study of Kremer and coworkers[21] there was no significant difference between 'high' (90 mg n-3 PUFA/kg/day) and 'low' (45 mg n-3 PUFA/kg/day) dose fish oil EE supplementation to decrease mononuclear LTB4 synthesis. The results of our study reveal that this may be explained by diminished bioavailability of the EE preparation. It is suggested that in 'dose finding' studies, the n-3 PUFA should be applied in TG form.

ACKNOWLEDGEMENTS

We are indebted to Ms I.K. Boomgaardt, Ms M van der Molen, Mr G Meiborg, Ms W. Wassenaar and Ms J. Wever.

REFERENCES

1. Kremer JM, Jubiz W, Michalek AV, et al. Fish oil fatty acid supplementation in active RA. Ann Intern Med 1987;106:497-503.
2. Tempel van der H, Tulleken JE, Limburg PC, Muskiet FAJ, Rijswijk van MH. Effects of fish oil supplementation in RA. Ann Rheum Dis 1990;49:76-80.

3. Tulleken JE, Limburg PC, Muskiet FAJ, Rijswijk van MH. The beneficial effects of dietary fish oil supplementation in RA cannot be ascribed to the added amount of alpha-tocopherol per se. Arthritis Rheum 1990,33:1416-1419.
4. Cleland LG, French JK, Betts WH, Murphy GA, Elliot MJ. Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. J Rheumatol 1988;15:1471-1475.
5. Endres S, Ghorbani R, Kelley VE, et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 1989;320:265-271.
6. Sperling I, Weinblatt M, Robin JL, et al. Effects of dietary supplementation with marine fish oil on leukocyte lipid mediator generation and function in RA. Arthritis Rheum 1987;30:988-997.
7. Gallon LS, Barcellu UO. Measurement of prostaglandin E3 and other eicosanoids in biologic samples using high pressure liquid chromatogrpahy and radioimmunoassay. Prostaglandins 1986;31:217-225.
8. Lawson LD, Hughes BG. Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethylesters co-ingested with a high fat meal. Biochem Biophys Res Commun 1988;156:960-963.
9. Reicks M, Hoadley J, Satchithanandam S, Morehouse KM. Recovery of fish oil-derived fatty acids in lymph of thoracic duct-cannulated Wistar rats. Lipids 1990;25:6-10.
10. Arnet FC, Edworthy SM, Bloch DA, et al. The American rheumatism association 1987 revised criteria for the classification of RA. Arthritis Rheum 1988;31:315-324.
11. Ritchie DM, Boyle JA, McInnes JM, et al. Clinical studies with an articular index for the assessment of joint tenderness in patients with RA. Quart J Med 1968;37:393-406.
12. Leeuwen van MA, Westra J, Limburg PC, Jong de HJ, Marrink J, Rijswijk van MH. Quantitation of IgM, IgA and IgG rheumatoid factors by ELISA in rheumatoid arthritis and other Rheumatoid Disorders. Scand J Rheumatol 1988;75:25-31.
13. Jansen S, Limburg PC, Bijzet J, et al. SAA versus CRP in chronic inflammatory diseases. Prot Biol Fluids 1986;34:347-350.
14. Clauss A. Gerinnungsphysiologische schnellmethode zur bestimmung des fibrinogen. Acta Haematol (Basel) 1957;17:237-246.
15. Verhagen J, Walstra P, Veldink GA, Vliegenthart JFG, Bruynzeel PLB. Separation and quantitation of leukotrienes by reversed phase high performance liquid chromatography. Prostaglandins Leukotrienes Med 1984;13:15-20.
16. Steege van der G, Muskiet FAJ, Martini IA, Hutter NH, Boersma ER. Simultaneous quantification of total medium- and LC fatty acids in human milk by capillary gas chromatography with split injection. J Chromatogr Biomed Appl 1987;415:1-11.
17. Hoving EB, Jansen G, Volmer M, Doormaal van JJ, Muskiet FAJ. Profiling of plasma cholesterol ester and triglycerid fatty acids as their methyl esters by capillary gas chromatography, preceded by a separation of lipid classes. J Chromatogr Biomed Appl 1988;434:395-409.
18. Zijlstra JG, Vries de JG, Muskiet FAJ, Martini IA, Timmer-Boscha H, Mulder NH. Influence of docosahexaenoic acid in vitro on intracellular adriamycin-sensitive and -resistant small-cell lung cancer cell lines, and on cytotoxicity in the tumor cell lines. Int J Cancer 1987;40:850-856.
19. Hoving EB, Prins J, Rutgers HM, Muskiet FAJ. Behavior of plasmalogens during high performance liquid chromatography on a silica column and a phosphoric acid containing mobile phase. J Chromatogr Biomed Appl 1988;434:411-416.
20. Muskiet FAJ, Doormaal van JJ, Martini IA, Wolthers BG, Slik van der W. Capillary gas chromatographic profiling of total LC fatty acids and cholesterol in biological materials. J Chromatogr Biomed Appl 1983;278:231-244.
21. Kremer JM, Lawrence DA, Jubiz W, et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Arthritis Rheum 1990;33:810-820.
22. Lawson D, Hughes BG. Human absorption of fish oil fatty acids as triacylglycerols, free fatty acids, or ethyl esters. Biochem Biophys Res Comm 1988;152:328-335.
23. Hamazaki T, Urakaze M, Makuta M, et al. Intake of different eicosapentaenoic acid contaning lipids and fatty acid pattern of plasma lipids in the rats. Lipids 1987;22:994-998.
24. Montfoort A, van der Werf L, Hartog JM, et al. The influence of fish oil diet and norepinephrine treatment on fatty acid composition of rat heart phospholipids and the positional fatty acid distribution in phosphatidylethanolamine. Basic Res Cardiol 1986;81:289-302.

25. Lee TH, Hoover RL, Williams JD, et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 1985;312:1217-1224.
26. Strasser T, Fischer S, Weber PC. Leukotriene B5 is formed in human neutrophils after dietary supplementation with eicosapentaenoic acid. Proc Natl Acad Sci (USA) 1985;82:1540-1543.
27. Bradlow BA, Chetty N, Westhuysen van der J, Mendelsohn D, Gibson JE. The effects of a mixed fish diet on platelet function, fatty acids and serum lipids. Thromb Research 1983;29:561-568.
28. Nagakawa Y, Oriino H, Harasawa M, Morita I, Yashira K, Murota S. Effect of eicosapentaenoic acid on the platelet aggregation and composition of fatty acid in man. Atherosclerosis 1983;47:71-75.
29. Shacky von C, Weber PC. Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. J Clin Invest 1985;76:2446-2450.
30. Anonymous. Arachidonate retention in essential fatty acid deficiency. Nutr Rev 1986;44:213-215.
31. Garg ML, Sebokova E, Thomson ABR, Clandinin MT. Delta 6 Desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or omega-3 fatty acids. Biochem J 1988;249:351-356.
32. von Schacky C, Weber PC. Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. J Clin Invest 1985;76:2446-2450.
33. Hagve T, Christophersen BO. Evidence for peroxismal retroconversion of adrenic acid (22:4(n-6)) and docosahexaenoic acids (22:6(n-3)) in isolated liver cells. Biochim Biophys Acta 1986:165-173.

Chapter 6

THE EFFECT OF DIETARY ENERGY PERCENTAGE OF FAT AND ITS P/S RATIO ON INCORPORATION OF N-3 PUFA AND LEUKOTRIENE PRODUCTION DURING FISH OIL SUPPLEMENTATION IN HEALTHY VOLUNTEERS

Jacob E. Tulleken, Pieter C. Limburg, Frits A.J. Muskiet,
Karin M. Kazemier, Immie K. Boomgaardt, Martin H. van Rijswijk

SUMMARY

The effect of low (25% of energy) and high (35% of energy) fat diets with either low (<0.4) or high (>1.0) polyunsaturated/saturated fatty acid (P/S) ratios on fatty acid compositions of plasma cholesterol esters and neutrophil phospholipids, and leukotriene production was studied in 4 groups of healthy volunteers supplemented with 6 g fish oil daily for 4 weeks. Except for three subjects, eicosapentaenoic acid (20:5,n-3) content markedly increased from baseline in the plasma cholesterol ester fraction and to a lesser extent in the neutrophil phospholipid fraction. The increase in the plasma cholesterol ester fraction was inversely related to the dietary intake of linoleic acid. At supplementation endpoints, the 20:5,n-3/20:4,n-6 ratio in both plasma cholesterol esters and neutrophil phospholipids was highest in the groups consuming diets with a low P/S ratio. In vitro leukotriene B5 production by neutrophils, did not differ between groups and there was no consistent suppression of LTB4 production in this 4 week study. It is suggested that reduction of the dietary linoleic acid intake during fish oil supplementation provides possible means to facilitate the accumulation of 20:5,n-3 in the plasma cholesterol ester fraction.

INTRODUCTION

Dietary fish oil supplementation improves joint symptoms in patients with rheumatoid arthritis[1-5]. Eicosapentaenoic (20:5,n-3) and docosahexaenoic (22:6,n-3) acids are the main long chain n-3 polyunsaturated fatty acids (n-3 PUFA) in fish oil. By virtue of increasing the 20:5,n-3 and 22:6,n-3 contents of cell membrane phospholipids dietary n-3 PUFA competitively inhibit the formation of arachidonic acid (20:4,n-6) derived cyclooxygenase metabolites like thromboxane A2[6] (TXA2) and prostaglandin E2[7] (PGE2). In the lipoxygenase pathway it has been shown that fish oil supplementation leads to decreased leukotriene B4 (LTB4) production[1,2,4,8] and thereby affects LTB4 mediated reactions like: macrophage interleukin-1 production[4,7] and chemotaxis and adhesion of both neutrophils[8] and monocytes[9]. The shift in the synthesis of prostaglandins and leukotrienes may be useful in patients with chronic inflammatory disorders, like rheumatoid arthritis.

PUFA of the n-3 series compete with n-6 PUFA for enzymes involved in fatty acid chain elongation, desaturation and eicosanoid synthesis. Animal studies have shown that the effect of fish oil supplementation on the synthesis of eicosanoids increases when combined with diets at low linoleic- (18:2,n-6) to saturated fatty acid ratios[10,11].

We studied the effect of low (25% of energy) and high (35%) fat diets at low (<0.4) or high (>1.0) polyunsaturated/saturated fatty acid (P/S) ratios on fatty acid compositions of plasma cholesterol esters (CE) and neutrophil phospholipids (PL), and leukotriene production in four groups of healthy volunteers during dietary fish oil supplementation.

MATERIALS AND METHODS

Participants, experimental diets and study design:

26 apparently healthy persons (13 females and 13 males) volunteered to participate in the study. There was no history of metabolic or chronic disease, present drug use or unusual dietary habbits. The subjects were randomly allocated to one of the following four dietary groups:

Group I: dietary fat= 25% of energy, P/S<0.4.
Group II: dietary fat= 25% of energy, P/S>1.0.
Group III: dietary fat= 35% of energy, P/S<0.4.
Group IV: dietary fat= 35% of energy, P/S>1.0.

A nutritionist estimated the subjects's usual dietary food intake by a 24 hour-recall method. Subsequently, we pursued to put each participant on an eucaloric dietary intake regimen which was calculated from the recommended energy intake for age, size and usual activity level, as advised by the Dutch recommended daily allowances[12]. The micronutrients for which food data are available were calculated to meet the criteria given in the same report. Reduction of the energy percentage of fat was compensated for by increasing that of carbohydrates, without concomitant changes in percentage energy from proteins.

After a three weeks run-in period, in which the participants adhered to their prescribed diets, they were additionally supplemented with 12 fish oil capsules per day, for four weeks. The fish oil capsules were generously provided by Aerofako, Amersfoort, The Netherlands. They mainly contained 20:5,n-3 (31 mol/100 mol fatty acids) and 22:6,n-3 (22 mol/100 mol fatty acids) in their ethyl ester form, resulting in a daily supply of 2.04 g 20:5,n-3 and 1.32 g 22:6,n-3 and a total of 6 g oil. Their total fatty acid composition is given in Table 1.

Blood sampling:

Morning fasting blood samples were collected at the start and at the end of the fish oil supplementation period. Standard laboratory tests were performed, including a complete blood cell count (Coulter counter, model S-plus III; Coulter Electronics Ltd, Luton Bedforshire, UK), serum total cholesterol and triglycerides by Sequential Multiple Analyzer plus Computer, (Technicon, Tarrytown, NY, USA) and high density lipoprotein-cholesterol (HDL-C) by Monotest (Boehringer Mannheim, Germany) after precipitation of low density lipoprotein cholesterol (LDL-C) and very low density lipoproteins by a mixture of magnesium acetate and phospotungstic acid. LDL-C lev-

els were calculated[13]. The apolipoproteins (Apo-A1 and Apo-B) were determined by an immunoturbidimetric method (Boehringer Mannheim, Germany) using a Multi-Stat-I centrifugal analyzer (Instrumentation Laboratories, IJselstein, The Netherlands).

Table 1 Fatty acid composition (mol/100 mol) of the fish oil supplement.

SAFA	7.68
8:0	0.01
10:0	0.12
12:0	0.02
14:0	0.49
16:0	2.65
18:0	3.86
20:0	0.38
22:0	0.13
MUFA	24.98
16:1,n-7	0.81
18:1,n-7	4.26
20:1,n-7	0.50
18:1,n-9	9.40
20:1,n-9	4.34
22:1,n-9	0.68
24:1,n-9	0.51
22:1,n-11	14.48
PUFA	67.34
18:3,n-3	1.02
18:4,n-3	3.37
20:4,n-3	1.99
20:5,n-3	31.68
22:5,n-3	3.80
22:6,n-3	21.51
18:3,n-6	0.16
18:2,n-6	1.51
20:2,n-6	0.45
20:3,n-6	0.31
20:4,n-6	0.99
22:4,n-6	0.16
22:5,n-6	0.39
n-3	63.37
n-6	3.97
n-9	14.93
n-11	14.48

Assessment of in vitro production of leukotrienes by neutrophils:
Neutrophils were isolated from 30 ml of peripheral blood and resuspended to a final concentration of 10^7 cells/mL. After stimulation with 10 uM calciumionophore A-23187 the released leukotrienes were measured by high performance liquid chromatography using a C18 column[2].

Determination of neutrophil PL fatty acid composition:
Neutrophil lipids were extracted with chloroform/methanol. The total phospholipid fraction was isolated by HPLC, essentially as described previously[15,16]. The fatty acid composition was determined by capillary gas chromatography using an apolar stationary phase[17].

Determination of plasma CE fatty acid composition:
After preparation of a plasma total lipid extract and isolation of the CE fraction by amino propylsilica columns, its fatty acid composition was determined by capillary gas chromatography[18].

Statistical analysis:
Data are presented as median (range), unless otherwise indicated. To study the change from baseline, we used the Wilcoxon test for paired data. The Kruskall-Wallis one way analysis of variance was used to compare the between group differences at baseline and after treatment and to compare the between group changes from baseline. The p value for each of the response variables was considered significant if less than 0.05. The Spearman correlation coefficient was calculated to investigate relationships between variables.

RESULTS

The groups were comparable with regard to age, height and body weight. (Table 2.) This table also shows the daily energy intake and the energy percentages from carbohydrates, fat and protein, not including the fish oil supplement, during the study. All persons completed the 7 weeks study. At the end of the study, body weight of subjects in groups I and II, consuming the low fat diets (25% of energy) decreased from 75 (63.5-90) to 71 (61-86) kg, $p<0.01$. There was no change in body weight in the subjects of groups III and IV (from 70.5 (57.5-93) kg at the beginning to 70.5 (57.5-88) kg at the end of the study) who consumed the high fat diets (35% of energy).

Table 2 Subject characteristics and dietary composition, not including the fish oil supplement, of the four groups during the seven-week study.

	I (25%, P/S<0.4)	II (25%, P/S>1.0)	III (35%, P/S<0.4)	IV (35%, P/S>1.0)
Participants (n)	6	6	8	6
Age (years)	29 (23-34)	29 (21-34)	29 (24-53)	35 (30-42)
Height (cm)	174 (165-185)	185 (162-189)	179 (166-189)	178 (178-181)
Weight (kg)	73.5 (67-85.5)	78 (63.5-90)	70.3 (57.5-93)	71 (61-85)
Energy (kJ)	5420 (4330-7690)	6086 (5170-9260)	9920 (6890-10780)	7730 (7140-9500)
Carbohydr.(%)	51 (48-60)	52 (50-57)	42 (37-52)	46 (44-52)
Fat (%)	26 (25-27)	25 (23-26)	37 (37-40)	35 (33-37)
Protein (%)	23 (14-27)	20 (20-25)	16 (11-20)	15 (9-18)
Alcohol (%)	0 (0-4)	2 (0-5)	3 (0-11)	0.4 (0-4)
Linoleic acid (g)	5 (4-8)	15 (11-22)	10 (6-13)	25 (20-32)
P/S*	0.4 (0.3-0.5)	1.1 (1-2)	0.3 (0.2-0.4)	1 (1-1.1)

Age, height and weight were recorded at the beginning of the study. * P/S denotes the molar ratio of dietary polyunsaturated/saturated fatty acids.

The effects of dietary fish oil supplementation on plasma lipid levels are summarized in Table 3. Plasma triglyceride concentrations decreased in three groups although only

Table 3 Effects of fish oil supplementation on serum lipids and apolipoproteins.

	I	II	III	IV
Cholesterol (mmol/L)				
before	3.5 (2.8-5.2)	5.5 (3.4-6.7)	5.4 (4.3-7.1)	4.9 (4.0-5.2)
after	4.2 (3.2-6.2)*	5.2 (3.8-6.2)	5.1 (4.1-7.9)	4.5 (4.1-5.4)
HDL-C (mmol/L)				
before	1.1 (0.9-1.6)	1.1 (0.9-1.8)	1.2 (0.8-1.6)	1.1 (0.9-1.5)
after	1.1 (0.7-1.8)	0.9 (0.7-1.2)	1.2 (0.9-2.4)	1.2 (0.9-1.6)
LDL-C (mmol/L)				
before	2.0 (1.4-2.9)	3.4 (1.9-4.6)	3.6 (2.8-4.7)	3.3 (2.7-3.4)
after	2.5 (1.8-3.9)**	3.7 (2.4-4.5)	3.6 (1.8-6.0)	2.9 (2.5-3.9)
Triglycerides (mmol/L)				
before	0.9 (0.5-1.7)	1.2 (0.9-2.2)	1.1 (0.7-3.2)	0.9 (0.7-1.7)
after	0.8 (0.5-0.9)	1.2 (0.6-2.0)	0.8 (0.6-1.5)**	0.7 (0.7-0.9)
Apo-A1 (g/L)				
before	1.4 (1.2-1.6)	1.6 (1.3-1.8)	1.5 (1.5-1.5)	1.3 (1.3-1.4)
after	1.5 (1.4-1.9)	1.4 (1.3-1.6)	1.6 (1.6-1.9)	1.4 (1.3-1.4)
Apo-B (g/L)				
before	0.5 (0.3-0.6)	0.7 (0.6-1.0)	0.7 (0.5-1.1)	0.7 (0.5-0.8)
after	0.5 (0.4-0.7)	0.7 (0.5-0.8)	0.7 (0.4-1.0)	0.5 (0.5-0.7)

* $p< 0.05$; ** $p<0.03$; versus before dietary fish oil supplementation.

for group III the level of significance was achieved. Total plasma cholesterol was altered in group I, owing to its increase in the LDL fraction. HDL-C and apolipoproteins A1 and B were not altered.

Fatty acid composition of plasma cholesterol esters:
The fatty acid compositions of plasma CE are shown in Table 4. After treatment there was a significant rise in the relative amount of 20:5,n-3 in all groups, whereas

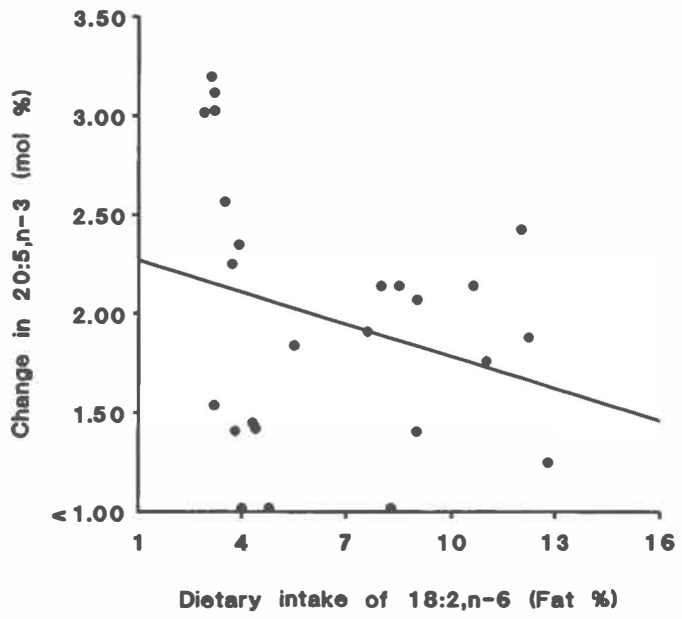
Table 4 Fatty acid composition (medians) of the plasma cholesterol ester fraction before and after four weeks of dietary fish oil supplementation (mol/100 mol).

Fatty acids		I	II	III	IV	Intergroup P
SAFA	before	17.2	13.1	15.3	16.9	
	after	19.2 •	17.8 •	17.7 •	17.8	<0.05
14:0	before	0.6	0.3	0.7	0.5	
	after	0.7	0.9 •	0.9	0.7	
16:0	before	14.2	11.4	12.8	14.2	
	after	16.9 •	15.5 •	15.2 •	15.4	<0.05
18:0	before	1.8	1.3	1.6	1.9	
	after	1.6	1.8	1.6	1.6 •	
MUFA	before	22.5	20.7	25.0	19.5	<0.05
	after	25.9 •	18.4	24.5	20.0	<0.05
16:1,n-7	before	2.7	2.7	3.5	2.2	
	after	3.4	2.5	3.0	2.6	
18:1,n-7	before	1.7	1.5	1.6	1.4	
	after	2.1 •	1.6	1.7 •	1.5	
18:1,n-9	before	17.3	17.0	19.5	15.9	<0.05
	after	20.2 •	14.6 •	19.9	16.0	<0.01
PUFA	before	62.3	66.2	59.3	63.4	
	after	55.4 •	63.7	56.9 •	61.9	<0.05
18:2,n-6	before	52.3	55.6	52.0	56.9	
	after	46.1 •	54.6	49.4 •	53.4	<0.01
18:3,n-6	before	0.5	0.6	0.6	0.5	
	after	0.2 •	0.4 •	0.3 •	0.3	
20:3,n-6	before	0.7	0.7	0.7	0.7	
	after	0.5 •	0.5 •	0.4 •	0.4 •	
20:4,n-6	before	6.2	6.7	5.1	4.6	<0.05
	after	4.5 •	4.9 •	3.8 •	3.7	
18:3,n-3	before	0.5	0.4	0.7	0.4	<0.01
	after	0.5	0.4	0.6	0.3	<0.01
20:5,n-3	before	0.5	0.3	0.6	0.2	<0.05
	after	2.9 •	2.4 •	2.5 •	2.1 •	
22:6,n-3	before	0.5	0.5	0.6	0.6	
	after	0.7	0.7	0.6	0.5	
P/S	before	3.6	5.0	4.0	3.7	
	after	2.8 •	3.6 •	3.2 •	3.4	<0.05
n-3	before	1.5	1.3	2.1	1.2	<0.01
	after	4.0 •	3.5 •	3.8 •	3.0 •	
n-6	before	60.6	64.5	57.5	62.1	
	after	51.7 •	60.3 •	53.8 •	58.6	<0.01
n-7	before	4.4	4.0	5.1	3.5	
	after	5.6	4.2	4.7	4.0	
n-9	before	17.3	17.0	19.5	15.9	<0.01
	after	20.2 •	14.6 •	19.9	16.0	<0.01
DBI	before	1.6	1.7	1.6	1.6	
	after	1.6	1.7	1.6	1.6	
EPA/AA[1]	before	0.0	0.0	0.1	0.0	
	after	0.7 •	0.5 •	0.7 •	0.5 •	<0.01

Intergroup P: analysis of variance for the comparison between all groups. • $p<0.05$, versus before dietary fish oil supplementation. SAFA: saturated fatty acids, MUFA: monounsaturated fatty acids, DBI:double bond index, EPA/AA denotes 20:5,n-3/20:4,n-6. 1: data in mol/mol.

no significant change occurred in that of 22:6,n-3. The loss of body weigth as was observed in some subjects of group I and II had no impact on the increase of relative amounts of 20:5,n-3 in plasma CE during fish oil supplementation. In general, the rise of n-3 PUFA occurred at the expense of n-6 PUFA. Figure 1 shows the relationship between changes in the relative amounts of 20:5,n-3 in the plasma cholesterol ester fraction and the dietary intake of 18:2,n-6 (in energy % fat).

Figure 1
Relationship between changes in the relative amounts of 20:5,n-3 in the plasma cholesterol ester fraction at the end of the four weeks fish oil supplementation and the dietary intake of 18:2,n-6 expressed as energy% of fat. r=-0.410, p<0.05.



Fatty acid composition of neutrophil phospholipids:
The fatty acid composition of neutrophil phospholipids is shown in Table 5. Except for 20:2,n-6, between group comparison of data at baseline revealed no significant differences. Upon fish oil supplementation, a significant rise in 20:5,n-3 occurred in all groups. For 22:6,n-3 this occurred in group I only. At treatment endpoints the groups differed significantly in the relative amounts of 18:2,n-6 and 20:2,n-6, 22:5,n-3 and total n-6 PUFA, and in the 20:5,n-3/20:4,n-6 ratio.

Table 5 Fatty acid composition of the neutrophil total phospholipid fraction before and after four weeks of dietary fish oil supplementation (mol/100 mol).

Fatty acids		I	II	III	IV	Intergroup P
SAFA	before	53.6	59.1	57.3	59.0	
	after	54.4	63.3	60.9	56.6	
14:0	before	1.7	1.4	1.5	1.5	
	after	1.2	1.5	1.1	1.3	
16:0	before	31.1	33.1	32.8	34.4	
	after	30.6	32.7	34.8	31.8	
18:0	before	19.0	20.6	19.9	22.1	
	after	19.7	23.7	21.5	17.4	
MUFA	before	31.1	25.4	28.0	24.5	
	after	31.5	22.9	26.1	25.8	
18:1,n-7	before	2.6	1.5	2.4	2.0	
	after	2.7	1.8	2.0	1.7	
18:1,n-9	before	24.0	20.1	22.7	19.7	
	after	24.9	19.1	20.9	21.0	
PUFA	before	15.0	15.4	15.6	17.2	
	after	13.6	15.6	12.5	17.7	
18:2,n-6	before	6.9	6.9	6.7	9.8	
	after	6.2	7.6	6.0	10.1	
20:2,n-6	before	0.7	0.6	0.6	0.9	<0.05
	after	0.5 •	0.6	0.5	0.9	<0.05
20:3,n-6	before	0.7	0.9	0.9	0.8	
	after	0.6	0.7	0.6 •	0.7	
20:4,n-6	before	4.1	3.8	4.6	3.4	
	after	3.2 •	2.8	3.2 •	3.5	
22:4,n-6	before	0.8	0.8	0.9	0.7	
	after	0.5	0.5 •	0.4 •	0.5	
18:3,n-3	before	0.4	0.3	0.3	0.2	
	after	0.4	0.3	0.3	0.3	
20:5,n-3	before	0.2	0.0	0.1	0.1	
	after	0.6 •	0.4 •	0.5 •	0.5 •	
22:5,n-3	before	0.3	0.3	0.6	0.2	
	after	0.7	0.5	0.5	0.8	<0.05
22:6,n-3	before	0.3	0.5	0.5	0.3	
	after	0.5 •	0.7	0.5	0.5	
P/S	before	0.3	0.2	0.3	0.3	
	after	0.3	0.2	0.2	0.3	
n-3	before	1.3	1.0	1.4	0.7	
	after	2.0 •	1.9 •	1.9 •	2.0 •	
n-6	before	13.1	14.4	14.0	16.6	
	after	11.5	13.5	10.5	15.6	<0.05
DBI	before	0.8	0.7	0.8	0.7	
	after	0.7	0.7	0.6	0.8	
EPA/AA[1]	before	0.0	0.0	0.0	0.0	
	after	0.2	0.1	0.2	0.1	<0.05

Intergroup P: analysis of variance for the comparison between all groups. • $p<0.05$, versus before dietary fish oil supplementation. (For technical reasons the neutrophil PL fatty acid composition of group IV could be measured for 3 subjects only). SAFA: saturated fatty acids, MUFA: monounsaturated fatty acids, DBI:double bond index, EPA/AA denotes 20:5,n-3/20:4,n-6. 1: data in mol/mol.

Pooling of fatty acid data on the basis of dietary P/S ratio:

Figure 2 shows the individual relative amounts of 20:5,n-3 together with the individual 20:5,n-3/20:4,n-6 ratio for the plasma CE fraction before and after fish oil supplementation, when the fatty acid data were pooled on the basis of dietary P/S ratio. The relative amounts of 20:5,n-3 and the 20:5,n-3/20:4,n-6 ratio increased both in the pooled P/S<0.4 and in the P/S>1.0 group during fish oil supplementation. Between group

comparison revealed that the ratio was higher in the P/S<0.4 group after fish oil supplementation ($p<0.05$).

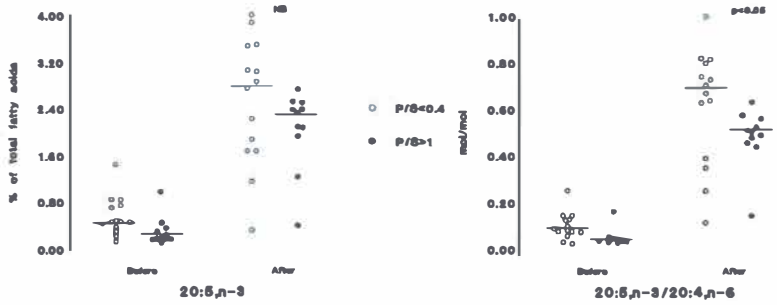


Figure 2 Individual relative amounts of 20:5,n-3 together with individual 20:5,n-3/20:4,n-6 ratios for the plasma cholesterol ester fraction before and after dietary fish oil supplementation. The data are derived from pooled values from persons consuming diets with P/S ratios of <0.4 (groups I+III) and >1.0 (groups II+IV). $p<0.05$, denotes the between group comparison of median after treatment values.

Figure 3 shows the individual relative amounts of 20:5,n-3 together with individual 20:5,n-3/20:4,n-6 ratios for the neutrophil PL fraction before and after fish oil supplementation, when the fatty acid data were pooled on the basis of dietary P/S ratio.

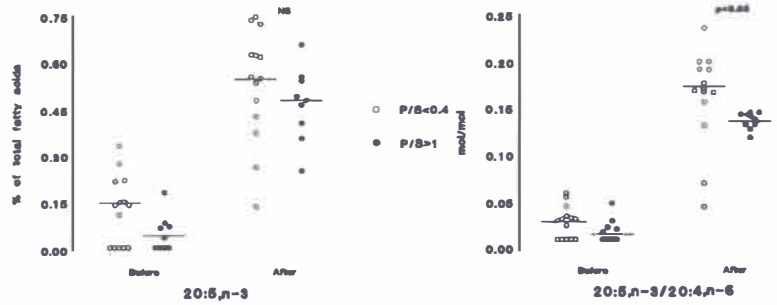


Figure 3 Individual relative amounts of 20:5,n-3 together with individual 20:5,n-3/20:4,n-6 ratios for the plasma neutrophil phospholipid fraction before and after fish oil supplementation. The data are derived from pooled values from persons consuming diets with P/S ratios of <0.4 (groups I+III) and >1.0 (groups II+IV). $p<0.05$, denotes the between group comparison of median after treatment values.

The relative amounts of 20:5,n-3 and the 20:5,n-3/20:4,n-6 ratio increased both in the pooled P/S<0.4 and in the P/S>1.0 group during fish oil supplementation. Between group comparison revealed that after treatment the 20:5,n-3/20:4,n-6 ratio was slightly higher in the PS<0.4 group (0.16), compared with the P/S>1.0 group (0.14) ($p<0.05$) When the groups were pooled by energy% fat no such trends were observed in neither the plasma CE fraction nor in the neutrophil PL fraction (data not shown).

In vitro eicosanoid production:

The effect of fish oil supplementation on in vitro leukotriene production by neutrophils in the different groups is shown in Table 6. The formation of LTB4 was suppressed in most groups, but significant differences were only achieved in groups I and IV. In all groups, a similar, significant, rise in the in vitro LTB5 production was noted during fish oil supplementation.

Table 6 Neutrophil leukotriene production before and after four weeks of dietary fish oil supplementation.

	I	II	III	IV
Leukotriene B4				
before	159.0 (142.0-205.0)	174.0 (150.0-221.0)	159.0 (106.0-205.0)	174.0 (152.0-210.0)
after	137.0 (100.0-157.0)*	186.0 (123.0-221.0)	127.0 (111.0-221.0)	137.0 (103.0-168.0)*
Leukotriene B5				
before	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
after	8.0 (5.0-9.0)**	6.0 (2.0-12.0)**	9.0 (5.0-15.0)**	6.0 (2.0-9.0)**
Leukotriene B5/leukotriene B4				
before	0.0 (0.00-0.00)	0.0 (0.00-0.00)	0.0 (0.00-0.00)	0.0 (0.0-0.0)
after	0.05 (0.03-0.07)	0.04 (0.01-0.06)	0.05 (0.03-0.06)	0.05 (0.03-0.06)

Leukotrienes are presented as ng/10^7 neutrophils. * $p<0.05$, ** $p<0.01$, versus before dietary fish oil supplementation.

Pooling of eicosanoid data on the basis of dietary P/S ratio revealed that after treatment, the LTB5/LTB4 ratio tended to be higher in the PS<0.4 groups (0.07 (0.02-0.11)) than in the in the PS>1.0 group (0.04 (0.00-0.06)), although the difference was not significant ($p=0.07$).

DISCUSSION

We studied the laboratory effects of dietary fish oil supplementation in healthy volunteers consuming diets at different energy percentage fat and P/S ratios. The results demonstrate that the increase in the relative amounts of 20:5,n-3 in plasma CE fraction is inversely related to the dietary intake of 18:2,n-6. and that the 20:5,n-3/20:4,n-6 ratios in plasma CE and neutrophil PL are higher in subjects consuming the low (<0.4) P/S diets, when compared with those consuming the high (>1.0) P/S diets. These results corroborate the conclusions of Sawazaki et al[19] and those drawn from some animal studies[10,11].

The inverse relationship of n-3 and n-6 PUFA incorporation in body lipids is the result of competition of these fatty acids for enzymes that catalyze the synthesis of cell and plasma lipids. For example in the liver, 20:5,n-3 competes with 18:2,n-6 for

the sn-2 position of locally synthesized phosphatidylcholine[20]. When the latter is secreted from the liver in the form of lipoproteins it serves as a substrate for the lecithin-cholesterol acyltransferase (LCAT) mediated transesterification of cholesterol in plasma. Competition between dietary 18:2,n-6 and 20:5,n-3 may additionally occur at the level of intestinal acyl-CoA cholesterol acyltransferase (ACAT), which produces CE from intestinally absorbed cholesterol. These CE are added to the plasma when chylomicrons reach the circulation.

It should, however, be emphasized that the dependency of 20:5,n-3 incorporation on dietary 18:2,n-6 is weak and differences in 20:5,n-3/20:4,n-6 ratio between the low and high P/S groups were small. Moreover, three persons failed to show an increase in the relative amounts of 20:5,n-3 in the plasma CE fraction. The latter is considered to reflect the dietary fatty acid composition consumed during the last three weeks[21]. It therefore seems that other factors additionally influence 20:5,n-3 incorporation during fish oil supplementation. One of these may be the efficacy of intestinal absorption of n-3 PUFA which is subject to larger variation when administered in its ethyl ester form (as in this study) than in its natural triglyceride form[22,23,24]. Diminished intestinal absorption may have hampered accumulation of 20:5,n-3 in plasma CE in three subjects (Figure 1). A second factor that may affect the accumulation of 20:5,n-3 in cell and plasma lipids is the large amount of previously absorbed 18:2,n-6 that is stored in adipose tissue. This pool becomes mobilized in the fasting state. It has been e.g. demonstrated that prevention of free fatty acid mobilization by an 48 hour continuous enteral hyperalimentation with carbohydrates alone leads to a decrease of plasma and erythrocyte 18:2,n-6[25]. Persons living in industrialized countries consume high amounts of 18:2,n-6 and relatively small amounts of n-3 PUFA, which consequently leads to a high 18:2,n-6/n-3 PUFA ratio in their adipose tissue. The turnover rate of fatty acids in this large triglyceride pool is about 300-600 days[26], which implies that a change in dietary fat intake will not accomplish a new steady state within about three years. Both the actual intake of 18:2,n-6 and the large 18:2,n-6 pool in adipose tissue may help to explain why in the, mostly short term, high dose fish oil supplementation studies the relative amounts of 20:5,n-3 and 20:4,n-6 in plasma and cell lipid have rarely reached the level as measured in Eskimo natives[27]. The latter 'consume' a high n-3/n-6 PUFA ratio for a life time period.

The increase of relative amounts of 20:5,n-3 in the neutrophil PL fraction was less pronounced compared to the increase in the plasma CE fraction. It might be argued that a 4 weeks fish oil supplementation period is too short to obtain appreciable alterations in the fatty acid composition of neutrophil PL. However, at the end of the 4 weeks treatment period the relative amounts of 20:5,n-3 in neutrophil PLs proved comparable with those found in RA patients during a 12 weeks supplementation period[2]. One might speculate that, in comparison with plasma CE, at least in short term trials, the neutrophil PL fraction is relatively resistent to incorporation of 20:5,n-3 and that the neutrophil preferentially incorporates n-6 PUFA. It is also possible that n-3 PUFA slowly incorporate into the fatty acid pool that serves as a reservoir to the synthesis of neutrophil membrane phospholipids during cell proliferation and maturation. Elucidation of the underlying mechanism is of importance to achieve optimal effects of fish oil supplementation in e.g. RA, since the relative amounts of 20:4,n-6

and 20:5,n-3 reflect precursor availability for the production of LTB4 and LTB5, respectively.

In this trial dietary fish oil has been shown to have a moderate suppressing effect on LTB4 synthesis in most groups, while enhancing the production of small amounts of LTB5. The lack of clear differences in leukotriene production between the experimental groups is not surprising in view of the observed changes in the fatty acid composition of neutrophil PL.

In summary, dietary intake of 18:2,n-6 appears to interfere with the accumulation of 20:5,n-3 in the plasma CE fraction during fish oil supplementation in healthy volunteers. Further research is warranted to establish a desirable ratio of dietary n-3/n-6 PUFA, to affect eicosanoid production and thereby eicosanoid mediated reactions.

ACKNOWLEDGEMENTS

We are indebted to Ms M. van der Molen, Mr G. Meiborg, Ms W.Wassenaar and Ms J. Wever.

REFERENCES

1. Kremer JM, Jubiz W, Michalek AV, et al. Fish oil fatty acid supplementation in active rheumatoid arthritis. Ann Intern Med 1987;106:497-503.
2. Tempel vd H, Tulleken JE, Limburg PC, Muskiet FAJ, Rijswijk van MH.: Effects of fish oil supplementation in rheumatoid arthritis. Ann Rheum Dis 1990;49:76-80.
3. Tulleken JE, Limburg PC, Muskiet FAJ, van Rijswijk MH. Vitamin E status during dietary fish oil supplementation in rheumatoid arthritis. Arthritis Rheum, 1990;33:1416-1419.
4. Kremer JM, Lawrence DA, Jubiz W, et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Arthritis Rheum 1990;33:810-820.
5. Cleland LG, French JK, Betts WH, Murphy GA, Elliot MJ. Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. J Rheumatol 1988;15:1471-1475.
6. Knapp HR, FitzGerald GA. The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. N Eng J Med 1989;320:1037-1043.
7. Endres S, Ghorbani R, Kelly VE, et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Eng J Med 1989;320:265-271.
8. Sperling I, Weinblatt M, Robin JL, et al. Effects of dietary supplementation with marine fish oil on leukocyte lipid mediator generation and function in rheumatoid arthritis. Arthritis Rheum 1987;30:988-997.
9. Schmidt EB, Nielsen LK, Pedersen JO, Kornerup HJ, Dyerberg J. The effect of n-3 polyunsaturated fatty acids on lipids, platelet function, coagulation, fibrinolysis and monocyte chemotaxis in patients with hypertension. Clin Chim Acta 1990;189:25-32.
10. Garg ML, Wierzbicki AA, Thomson ABR, Clandinin MT. Fish oil reduces cholesterol and arachidonic acid content more efficiently in rats fed diets containing low linoleic acid to saturated fatty acid ratios. Biochim Biophys 1988;962:337-344.
11. German JB, Lokesh B, Kinsella JE. The effect of dietary fish oils on eicosanoid biosynthesis in peritoneal macrophages is influenced by both dietary n-6 polyunsaturated fats and total dietary fat. Prostaglandins Leukotrienes and Essential Fatty Acids 1988;34:37-45.
12. Eekelen van M, Jansen BCP, Straub J. Nederlandse voedingsmiddelen tabel. Zeist 1987,35e druk
13. Friedewald WT, Levy RI,. Frederickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

14. Verhagen J, Walstra P, Veldink GA, Vliegenthart JFG, Bruynzeel PLB. Separation and quantitation of leukotrienes by reversed phase high performance liquid chromatography. Prostaglandins Leukotrienes Med 1984;13:15-20.
15. Zijlstra JG, Vries de JG, Muskiet FAJ, Martini IA, Timmer-Boscha H, Mulder NH. Influence of docosahexaenoic acid in vitro on intracellular adriamycin-sensitive and -resistant small-cell lung cancer cell lines, and on cytotoxicity in the tumor cell lines. Int J Cancer 1987;40:850-856.
16. Hoving EB, Prins J, Rutgers HM, Muskiet FAJ. Behavior of plasmalogens during high performance liquid chromatography on a silica column and a phosphoric acid containing mobile phase. J Chromatogr Biomed Appl 1988;434:411-416.
17. Muskiet FAJ, Doormaal van JJ, Martini IA, Wolthers BG, Slik van der W. Capillary gas chromatographic profiling of total LC fatty acids and cholesterol in biological materials. J Chromatogr Biomed Appl 1983;278:231-244.
18. Hoving EB, Jansen G, Volmer M, Doormaal van JJ, Muskiet FAJ. Profiling of plasma cholesterol ester and triglycerid fatty acids as their methyl esters by capillary gas chromatography, preceded by a separation of lipid classes. J Chromatogr Biomed Appl 1988;434:395-409.
19. Sawazaki S, Hamazaki T, Yamazaki K, et al. Comparison of the increment in plasma eicosapentaenoate concentrations by fish oil intake between young and middle-aged volunteers. J Nutr Sci Vitaminol 1989;35:349-359.
20. Holub BJ, Bakker DJ, Shaeff. Alterations in molecular species of cholesterol esters formed via plasma lecithine-cholesterol acyltransferase in human subjects consuming fish oil. Atherosclerosis 1987;66:11-18.
21. Moilanen T, Rasanen L, Vikari J, Akerblom HK, Ahola M, Uhari M, Passanen M, Nikkari T Fatty acid composition of serum cholesteryl ester in 3- to 18-year old Finnish chldren and its relation to diet. Am J Clin Nutr 1985;42:708-713.
22. Lawson LD, Hughes BG. Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethylesters co-ingested with a high fat meal. Biochem Biophys Res Commun 1988;156:960-963.
23. Lawson D, Hughes BG. Human absorption of fish oil fatty acids as triacylglycerols, free fatty acids, or ethyl esters. Biochem Biophys Res Comm 1988;152:328-335.
24. Reicks M, Hoadley J, Satchithanandam S, Morehouse KM. Recovery of fish oil-derived fatty acids in lymph of thoracic duct-cannulated Wistar rats. Lipids 1990;25:6-10.
25. Doormaal JJ, Muskiet FAJ, Martini IA, Doorenbos H. Rapid changes in serum, plasma and erythrocyte lipid compositions and serum transaminase levels during continuous enteral hyperalimentation by carbohydrates alone. Metabolism 1987;36:1132-1140.
26. Dayton S, Hashimoto, Dixon W, Pearce ML: Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. J Lipid Res 1966;7:103-111.
27. Kinsella JE, Lokesh B, Stone RA. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am J Clin Nutr 1990;52:1-28.

Chapter 7

LONG CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS IN RHEUMATOID ARTHRITIS

Jacob E. Tulleken, Pieter C. Limburg, Frits A.J. Muskiet,
Martin H. van Rijswijk

Polyunsaturated fatty acids:

Polyunsaturated fatty acids play important roles in the structural and metabolic functions of cellular membranes. Mono- and poly (PUFA) unsaturated fatty acids can be characterized by the number of carbon atoms from the last double bond up to and including the terminal methyl group of the carbon chain. PUFA with three (n-3 fatty acids) or six (n-6 fatty acids) carbon atoms in that position can not be *de novo* synthesized by the human body and hence are designated essential fatty acids.

Vegetable oils are main sources of dietary n-6 PUFA, of which linoleic acid (18:2,n-6) is the quantitatively most important species. Some terrestrial plants contain alpha-linolenic acid (18:3,n-3) in the chloroplast membrane but cold water fish and refined fish oils are the richest sources of n-3 PUFA. The main members of the n-3 PUFA series found in fish oils are eicosapentaenoic (EPA; designated 20:5,n-3) and docosahexaenoic (DHA; 22:6,n-3) acids.

Arachidonic acid (20:4,n-6) is an important constituent of cell membrane phospholipids (PL). Its dietary intake is low, since there are no major sources in regular foods. Cell membranes are therefore largely dependent upon dietary 18:2,n-6 as a precursor for the biosynthesis of 20:4,n-6 by microsomal desaturation and chain elongation.

Eicosanoids:

Eicosanoids is the designation for oxygenation products of long chain PUFA, of which 20:4,n-6 is the most important. Eicosanoids play an essential role in human hemostasis, immune response, and tissue perfusion but may also be involved in the pathogenesis of disease[1]. The formation of eicosanoids occurs through two different enzymatic pathways, i.e.: the cyclooxygenase pathway, leading to the production of prostaglandins, thromboxanes and prostacyclins; and the lipoxygenase pathway, leading to the production of leukotrienes. The rate-limiting step in the formation of eicosanoids is the release of 20:4,n-6 from membrane PL by a phospholipase. Activation with physiologic stimuli of different cell types leads to the activation of phospholipase A2. A similar effect occurs when cells are stimulated by an unphysiologic stimulus like calciumionophore A-23187[2].

In human leukocytes prostaglandin E2 (PGE2) is the predominant cyclooxygenase product of 20:4,n-6. PGE2 possesses both pro- and anti-inflammatory effects. It increases vascular permeability and blood flow at the site of inflammation, it induces fever, and has a synergetic activity with bradykinin in provoking pain. PGE2 inhibits cell proliferation and the production of interleukin-1 (IL-1)[3] and tumor necrosis factor (TNF)[4].

Leukotrienes of the 4 series are produced from 20:4,n-6 via the lipoxygenase pathway. They are potent lipid mediators which play an important role in allergic and inflammatory reactions[5]. For instance, leukotriene B4 (LTB4), mainly produced by neutrophils and monocytes, is a strong chemotactic agent[6]. Furthermore by potentiating the production of IL-1[7] and gamma-interferon[8,9] LTB4 is involved in the regulation of cytokine synthesis as well.

Dietary fish oil supplementation in rheumatoid arthritis:

With the increasing insight into the 20:4,n-6 metabolism it was obvious to investigate whether chronic inflammatory conditions, like rheumatoid arthritis (RA) can be influenced by adding a substantial amount of fish oil to the diet. In theory, its high conten of long chain n-3 PUFA could be a valuable supplement to the usual treatment modalities in RA. By virtue of increasing the 20:5,n-3 and 22:6,n-3 contents of cell membrane PL, dietary n-3 PUFA competitively inhibit the formation of 20:4,n-6 derived cyclooxygenase and lipoxygenase metabolites (PGE2 and LTB4) whereas the production of weak, n-3 PUFA derived metabolites, such as TXA3[10], PGE3[11] and LTB5[12,13] is enhanced.

Fish oils have been shown to suppress inflammation in both animals[14,15] and in patients with RA. In a placebo-controlled trial, Kremer et al.[16] were the first to demonstrate that the daily ingestion of fish oil capsules containing 2.7 g 20:5,n-3 and 1.8 g 22:6,n-3, resulted in a decrease of the mean time to the onset of fatigue and the number of tender joints in patients with RA. We reported a reduction of joint pain and swelling, as well as the duration of early morning stiffness during daily administration of 3.4 g of 20:5,n-3+22:6,n-3 for twelve weeks[17]. Others have reported similar beneficial clinical effects in controlled[18] and uncontrolled settings[19]. Recently it was demonstrated that improvement of joint symptoms was more common when RA patients were supplemented with 90 mg n-3 PUFA/kg/day compared with supplementation of 45 mg n-3 PUFA/kg/day[20]. The additional clinical benefit of the higher dosage was dissapointing, however, and there was no significant difference between the two dosages to decrease mononuclear LTB4 synthesis. This suggests that both dosages altered the fatty acid composition of the mononuclear membrane phospholipid fraction to the same extent since this fraction reflects precursor availability for leukotriene synthesis.

The fish oil fatty acids in the discussed studies were administered in the semi-synthetic ethyl ester (EE) form, which allows much higher concentrations of n-3 PUFA when compared with their relative amounts in the naturally occurring fish oil triglycerides (TG). However, recent studies have assessed a diminished absorption of EE preparations, compared with fish oil TG preparations[21,22]. If so, this may raise doubts about the therapeutic advantage of 'high' dose fish oil fatty acid EE over 'low' dose fish oil preparations in their natural TG form. In a twelve weeks, double blind study design we compared the clinical and laboratory effects of 'high' dose fish oil fatty acid EE (3.4 g 20:5,n-3+22:6,n-3/day) and 'low' dose fish oil TG preparations (1.7 g 20:5,n-3+ 22:6,n-3/day) in 29 patients with RA[23]. Both fish oil preparations were equally effective in the incorporation of 20:5,n-3 in plasma CE and neutrophil PL, inhibition of in vitro neutrophil LTB4 production and augmentation of LTB5 formation, and suppression joint of symptoms.

Thus, apart from the actual amount of fish oil fatty acids present in the capsules, the chemical form in which they are provided (EE or TG) may be of importance to the magnitude of the induced clinical and laboratory effects. It is suggested that in future 'dose finding' studies, the n-3 PUFA should be applied in TG form.

Dietary long chain n-3 PUFA interfere with n-6 PUFA chain elongation and desaturation by reducing delta-6 desaturase activity and thereby affecting the rate limiting step in the conversion of 18:2,n-6 to 20:4,n-6[24]. Analogously, we observed a decreased 20:3,n-6/18:2,n-6 ratio in the neutrophil PL fractions, suggesting diminished delta-6 desaturase and/or chain elongation activity. Surprisingly, the relative amounts of 20:4,n-6 in neutrophil PL only showed minor changes during dietary fish oil supplementation. Similar results were obtained by others[25-28] who showed that daily administration of 1 to 6 g fish oil did not seriously alter the relative amounts of 20:4,n-6 in neutrophils and platelets. However, not all studies are consistent in this respect. The fact that, despite diminished conversion of 18:2,n-6, the relative amounts of 20:4,n-6 remain stable may partially be explained by increased retroconversion of 22:4,n-6 into 20:4,n-6. Like 22:6,n-3 for 20:5,n-3, the 22:4,n-6 appears to be a reservoir for 20:4,n-6[29]. In animal studies, it has been established that there is marked retention of 20:4,n-6 in certain PL during essential fatty acid deficiency[30]. These data may indicate that the presence of 20:5,n-3 rather than the reduction of the 20:4,n-6 content in cell membrane PL is mainly responsible for the alterations in cell function during dietary fish oil supplementation.

In most human trials, dietary fish oil supplementation has been shown to induce a moderate reduction (about 20%) of the in vitro neutrophil LTB4 production, while the LTB5 production moderately increased from undetectable amounts up to 10% of LTB4. A major question regarding the leukotrienes is whether any of the induced alterations in their production contribute to the observed clinical improvements during fish oil supplementation. No correlations were found between changes in leukotriene production and alterations in clinical disease variables in this respect[20,17] although not all studies are consistent[16]. It seems unlikely that inhibition of the lipoxygenase pathway is the only cause of clinical improvement. As fish oil modulates the formation of both leukotrienes and prostaglandins, it is possible that the effects of fish oil may be a result of changes in the production of prostaglandins. Recently, the question has been raised whether the anti-inflammatory effects of fish oil are due to the action of n-3 PUFA or should in fact be ascribed to the addition of alpha-tocopherol only. The fish oil preparations have to be enriched with alpha-tocopherol to prevent peroxidation of their PUFA. It has been argued that the antioxidizing properties of alpha-tocopherol may interfere with the process of joint inflammation by reducing the free radical-mediated cellular lipid peroxidation. However, in a placebo controlled trial we have shown that the beneficial effects of fish oil supplementation cannot be ascribed to the encapsuled amount of alpha-tocopherol per se[31].

It is interesting to note that even during the use of non steroidal antiinflammatory drugs (NSAID), fish oil provides an additive beneficial effect. One of the mechanisms by which NSAID exert their effect is a strong inhibition of the production of prostaglandins whereas the production of leukotrienes remains unaffected. The ability of n-3 PUFA to

competitively inhibit both the production of cyclooxygenase- and lipoxygenase metabolites of 20:4,n-6 should, theoretically, provide the possibility to reduce NSAID demand in RA patients. Belch et al[32] have shown that treatment with evening primrose oil, rich in 18:3,n-6, with or without 20:5,n-3 (240 mg/day) did allow some RA patients to reduce, or even stop their usage of NSAID. Evening primrose oil raises the 20:3,n-6 content of cell membrane PL. It is the precursor of PGE1, a prostaglandin with antiinflammatory properties. The data from the combined treatment group (18:3,n-6+20:5,n-3) do not allow a conclusion with regard to the sole effect of 20:5,n-3 on the required dosage of NSAID. Therefore, we undertook a double blind, placebo-controlled trial to investigate whether fish oil consumption (3.4 g 20:5,n-3+22:6,n-3 for 12 weeks) could reduce the usage of indomethacin in patients with RA. Only minor effects on indomethacin demand was established for those patients who received fish oil, whereas no effects were observed in those patients who received placebo[33]. It is unclear why the relief of joint pain during the use of indomethacin cannot be mimicked by the use of fish oil. Recently, in healthy volunteers who ingested 4.6 g/day of 20:5,n-3+22:6,n-3 for six weeks, a 51% decrease of PGE2 release was measured in the supernatant of stimulated mononuclear cells[34]. It is possible that the fish oil-induced reduction of the PGE2 production is not sufficient to achieve similar clinical effects as observed during the use of a strong cyclooxygenase inhibitor like indomethacin. However, referring to the fact that indomethacin exerts some non prostaglandin dependent effects as well[35], it is also possible that fish oil does not exert such effects and hence cannot replace indomethacin. What ever the precise reason, one might conclude that the used treatment schedule of fish oil cannot lessen the need for NSAID in RA. This may raise the question whether fish oil has 'disease modifying' properties? Fish oil has the rather unique quality to inhibit the 20:4,n-6 derived products of the lipoxygenase pathway. Since leukotrienes are actively involved in inflammation, a reduced LTB4 production may have consequences for the processes of joint destruction in RA. By virtue of reducing the LTB4 production, dietary fish oil supplementation leads to diminished chemotaxis and adhesion of neutrophils[19] and monocytes[36]. In addition, plasma levels of IL-1 correlate with erythrocyte sedimentation rate (ESR) levels in RA patients[37] and fish oil supplementation has been shown to suppress the macrophage IL-1 production[34,20]. Surprisingly, in none of the trials it was reported that the ESR, rheumatoid factor titer, or the plasma levels of hemoglobin and C-reactive protein were altered during fish oil supplementation. Thus, the clinical relevance of the fish oil induced inhibition of IL-1 and TNF production in RA patients remains to be established and, indeed, no objective evidence has been obtained that fish oil supplementation will favourably alter the course of the disease.

The results of the aforementioned short term period trials indicate that fish oil EE should be considered as a supplement to rather than a substitute for nowadays anti-rheumatic drugs. We suggest, however, that large studies over more extended periods are required to draw definitive conclusions on the ability of fish oil, preferrably in TG form, to favourably alter the progression and prognosis of the disease. Furthermore, animal studies have shown that the effect of fish oil supplementation on the synthesis of eicosanoids increases when combined with diets at low 18:2,n-6 to saturated fatty acid ratios[38].

If so, a discussion of the role of 18:2,n-6 on the efficacy of dietary fish oil supplementation to alter the fatty acid composition of cell and plasma lipids and consequently eicosanoid synthesis and mediated reactions as well may be useful.

Possible interactions between dietary 18:2,n-6 and 20:5,n-3:
The n-6 PUFA and n-3 PUFA compete for the same binding site on the sn-2 position of the glycerol moiety in tissue PL. In the liver, 20:5,n-3 competes with 18:2,n-6 for the sn-2 position of phosphatidylcholine[39] that serves as a substrate for the lecithin-cholesterol acyltransferase (LCAT) mediated formation of plasma CE. Competition between dietary 18:2,n-6 and 20:5,n-3 may also occur at the level of the acyl-CoA cholesterol acyltransferase (ACAT) mediated formation of CE in the intestine. Evaluating the biochemical effects of dietary fish oil supplementation during the consumption of different energy% fat and polyunsaturated/saturated fatty acid (P/S) ratios in healthy volunteers, we demonstrated that the change in the relative concentration of 20:5,n-3 in plasma CE is inversely related to the dietary intake of 18:2,n-6[40]. However, the dependency of 20:5,n-3 incorporation on dietary intake of 18:2,n-6 was weak. It therefore seems that other factors additionally influence incorporation during fish oil supplementation. One of these may be the chemical in form in which the fish oil fatty acids are provided (EE or TG). Secondly, besides competing with dietary 18:2,n-6, absorbed 20:5,n-3 will have to compete with a large amount of previously absorbed 18:2,n-6 that is stored in adipose tissue and that becomes mobilized in the fasting state. Persons living in industrialized countries consume high amounts of 18:2,n-6 and relatively small amounts of n-3 PUFA, which consequently leads to a high 18:2,n-6/n-3 PUFA ratio in their adipose tissue. The turnover rate of fatty acids in this large, triglyceride pool is about 300-600 days[41] which implies that changes in dietary fat intake will not accomplish a new steady state within a period of at least three years. Both the actual intake of 18:2,n-6 and the large 18:2,n-6 pool in adipose tissue may help to explain why in the mostly short term, high dose fish oil supplementation studies, the relative amounts of 20:5,n-3 and 20:4,n-6 in plasma and cell lipid have rarely reached the level as measured in Eskimo natives[42]. The latter 'consume' a high n-3/n-6 PUFA ratio for a life time period[43]. The increase of relative amounts of 20:5,n-3 in the neutrophil PL fraction during dietary fish oil supplementation is much less pronounced than changes in the plasma CE fraction[17]. We have observed that after 4 weeks of fish oil supplementation in healthy volunteers, the relative amounts of 20:5,n-3 in neutrophil PL were comparable to those achieved in patients with RA during supplementation with similar amounts of fish oil over a 12 weeks period[17]. One might speculate that the neutrophil PL fraction is, at least in short term studies, relatively resistent to incorporation of 20:5,n-3 and has a preference to incorporate n-6 PUFA over n-3 PUFA. On the other hand it is possible that n-3 PUFA slowly incorporate into the fatty acid pool that serves as a reservoir to the synthesis of neutrophil membrane phospholipids during cell proliferation and maturation. The elucidation of the underlying mechanism is of importance to the achievement of optimum effects of dietary fish oil supplementation in RA since the relative amounts of 20:4,n-6 and 20:5,n-3 in neutrophil PL reflect precursor availability for the synthesis of LTB4 and LTB5 respectively.

Side effects:
At the dosage levels used in most clinical studies, only short term, moderate discomforts were observed. Nausea, ructus, and diarrhea were mostly mentioned. However, some potentially harmful adverse reactions to high dietary fish oil intake have also been reported. These include increased airway hyperresponsiveness in aspirin intolerant asthma patients[44], exacerbation of induced arthritis in some rat strains[15] and metabolic deterioration in type II diabetes[45]. Thus, it is likely that some physiologic and pathologic processes may be sensitive to modulation by these compounds.

Furthermore, when prescribing fish oils emphasis should be given to the next points: First, large doses of n-3 PUFA, given as cod liver oil may induce vitamin A, and D toxicity and increase cholesterol ingestion. Second, currently used dosages (up to 10 g/day) would add 380 KJ/day to the usual dietary energy intake which could lead to unwanted obesity. Third, n-3 PUFA administration may enhance lipid peroxidation. The added amount of antioxidant, mostly alpha-tocopherol, may not be adequate. Fourth, one study described that daily ingestion of 135 g mackerel paste in healthy male volunteers increased total plasminogen activator inhibitor activity. This could be a risk for reinfarction in myocardial infarction patients[46]. Fifth, in some studies a platelet count fall was observed during fish oil supplementation[28]. Sixth, fish oil supplementation may induce prolonged bleeding times and reduced platelet function, consequently this may be associated with bleeding diathesis[28]. Seventh, it has been shown, recently, that daily ingestion of 2.1 g 20:5,n-3 for six weeks depressed phagocytosis and humoral responses (enzyme release and circulating immunoglobulin levels) in a human volunteer[47]. Blonk et al.[48] observed, however, no significant changes in the ability of leukocytes to kill staphylococcus aureus. Discussion is still going on this point. In general, it can be argued that some concern about the long term safety is warranted, especially in situations when fish oil supplements are used without medical supervision.

Conclusion:
In conclusion, dietary fish oil supplementation has been shown to provide an additive beneficial effect during the use of anti-rheumatic drugs and may thus be helpful to reduce joint pain in RA patients. In view of the described effects a complete remission of symptoms is not to be expected. Large studies over extended periods are required to draw definitive conclusions on the ability of fish oil to favourably alter the progression and prognosis of the disease. Until such studies have been carried out we suggest that fish oil should not be considered as a substitute for nowadays NSAID and slow acting anti-rheumatic drugs.

REFERENCES

1. Cannon PJ: Eicosanoids and the blood vessel wall. Circulation 1984,70:523-528.
2. Borgeat P, Samuelsson B. Arachidonic acid metabolism in polymorponuclear leukocytes: effects of ionophore A23187. Proc. Natl. Sci. USA 1979;5:2148-2152.
3. Kunkel SL, Chensue SW, Phan SH: Prostaglandins as endogenous mediators of interleukin 1 production. J Immunol 1986;136:186-192.
4. Farrar WL, Humes JL: The role of arachidonic acid metabolism in the activities of interleukin 1 and 2. J Immunol 1985;135:1153-1159.
5. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxines: Structures, biosynthesis, and biological effects. Science 1987;vol 237:1171-1175.
6. Ford-Hutchinson AW, Bray MA, DOIG MV, Shipley ME, Smith MJH: Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 1980;286:264-265.
7. Plezczynski MR, Lemaire I: Leukotrienes augment interleukin 1 production by human monocytes. J Immunol 1985;135:3958-3961.
8. Johnson HM, Torres BA: Leukotrienes: positive signals for regulation of gamma-interferon production. J Immunol 1984,132:413-416.
9. Pleszcynski MR, Bouvrette L, Gingras D, Girard M: Identification of interferon as the lymphokine that mediated leukotriene B4 induced immunoregulation. J Immunol 1987;139:513-517.
10. Knapp HR, FitzGerald GA: The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. N Eng J Med 1989;320:1037-1043.
11. Gallon LS, Barcellu UO.Measurement of prostaglandin E3 and other eicosanoids in biologic samples using high pressure liquid chromatogrpahy and radioimmunoassay. Prostaglandins 1986;31:217-225.
12. Strasser T, Fischer S, Weber PC. Leukotriene B5 is formed in human neutrophils after dietary supplementation with eicosapentaenoic acid. Proc Natl Acad Sci (USA) 1985;82:1540-1543.
13. Terano T, Salmon JA, Moncado S. Biosynthesis and biological activity of leukotriene B5. Prostaglandins 1984;27:217-232.
14. Leslie CA, Conte JM, Hayes KC, Cathcart ES. A fish oil diet reduces the severity of collagen induced arthritis after onset of the disease.Clin Exp Immunol 1988;73:328-332.
15. Mc Coll SR, Cleland LG, Whitehouse MW, Vernon-Roberts B. Effect of dietary polyunsaturated fatty acid (PUFA) supplementation on adjuvant induced polyarthritis in rats. J Rheumatol 1987;14:197-201.
16. Kremer JM, Jubiz W, Michalek AV, et al. Fish oil fatty acid supplementation in active rheumatoid arthritis. Ann Intern Med 1987;106:497-503.
17. Tempel vd H, Tulleken JE, Limburg PC, Muskiet FAJ, Rijswijk van MH: Effects of fish oil supplementation in rheumatoid arthritis. Ann Rheum Dis 1990;49:76-80.
18. Cleland LG, French JK, Betts WH, Murphy GA, Elliot MJ. Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. J Rheumatol, 1988;15:1471-1475.
19. Sperling I, Weinblatt M, Robin JL, et al. Effects of dietary supplementation with marine fish oil on leukocyte lipid mediator generation and function in rheumatoid arthritis. Arthritis Rheum, 1987;9:988-997.
20. Kremer JM, Lawrence DA, Jubiz W, Digiacomo R, Rynes R, Bartholomew LE, Sherman M: Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Arthritis Rheum 1990;33:810-820.
21. Lawson LD, Hughes BG. Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethylesters co-ingested with a high fat meal. Biochem Biophys Res Commun 1988;156:960-963.
22. Reicks M, Hoadley J, Satchithanandam S, Morehouse KM. Recovery of fish oil-derived fatty acids in lymph of thoracic duct-cannulated Wistar rats. Lipids 1990;25:6-10.
23. Tulleken JE, Limburg PC, Muskiet FAJ, Kazemier KM, Rijswijk van MH. A comparison between the

effects of fish oil fatty acid ethyl esters and fish oil triglycerides in rheumatoid arthritis. Submitted
24. Garg ML, Sebokova E, Thomson ABR, Clandinin MT. delta-6 Desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or n-3 fatty acids. Biochem J 1988;249:351-356.
25. Bradlow BA, Chetty N, Westhuysen van der J, Mendelsohn D, Gibson JE. The effects of a mixed fish diet on platelet function, fatty acids and serum lipids. Thromb Research 1983;29:561-568.
26. Lee TH, Hoover RL, Williams JD et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 1985;312:1217-1224.
27. Nagakawa Y, Orimo H, Harasawa M, Morita I, Yashira K, Murota S. Effect of eicosapentaenoic acid on the platelet aggregation and composition of fatty acid in man. Atherosclerosis 1983;47:71-75.
28. Shacky von C, Weber PC. Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. J Clin Invest 1985;76:2446-2450.
29. Hagve T, Christophersen BO. Evidence for peroxismal retroconversion of adrenic acid (22:4(n-6)) and docosahexaenoic acids (22:6(n-3)) in isolated liver cells. Biochim Biophys Acta 1986:165-173.
30. Anonymous. Arachidonate retention in essential fatty acid deficiency. Nutr Rev 1986;44:213-215.
31. Tulleken JE, Limburg PC, Muskiet FAJ, van Rijswijk MH. Vitamin E status during dietary fish oil supplementation in rheumatoid arthritis. Arthritis Rheum, 1990;33:1416-1419.
32. Belch JJF, Ansell D, Madhok R, O'Dowdd A, Sturrock RD: Effects of altering dietary essential fatty acids on requirements for non-steroidal anti-inflammatory drugs in patients with rheumatoid arthritis: a double blind placebo controlled study. Ann Rheum Dis 1988;47:96-104.
33. Tulleken JE, Limburg PC, Wassenaar W, Rijswijk van MH. Nonsteroidal antiinflammatory drug demand during fish oil treatment in rheumatoid arthritis. Submitted
34. Endres S, Ghorbani R, Kelly VE, Georgilis K, Lonneman G, Meer van der JWM, Cannon JG, Rogers TS, Klempner MS, Weber PC, Schaefer EJ, Wolff SM, Dinarello CA: The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Eng J Med 1989;320:265-271.
35. Abramson SB, Weissmann G. The mechanisms of action of non steroidal antiinflammatory drugs. Arthritis Rheum 1989;1:1-9.
36. Schmidt EB, Nielsen LK, Pedersen JO, Kornerup HJ, Dyerberg J: The effect of n-3 polyunsaturated fatty acids on lipids, platelet function, coagulation, fibrinolysis and monocyte chemotaxis in patients with hypertension. Clin Chim Acta 1990;189:25-32.
37. Eastgate JA, Wood NC, Di Giovine FS, Symons JA, Grinlinton FM, Duff GW: Correlation of plasma interleukin 1 levels with disease activity in rheumatoid arthritis. Lancet, 1988;I:706-709.
38. German JB, Lokesh B, Kinsella JE: The effect of dietary fish oils on eicosanoid biosynthesis in peritoneal macrophages is influenced by both dietary n-6 polyunsaturated fatty acids and total dietary fat. Prostaglandins Leukotrienes and Essential Fatty Acids 1988;34:37-45.
39. Holub BJ, Bakker DJ, Shaeff. Alterations in molecular species of cholesterol esters formed via plasma lecithine-cholesterol acyltransferase in human subjects consuming fish oil. Atherosclerosis 1987;66:11-18.
40. Tulleken JE, Limburg PC, Muskiet FAJ, Kazemier KM, Boomgaardt IK, Rijswijk van MH. The effect of dietary energy percentage of fat and its P/S ratio on incorporation of n-3 PUFA and leukotriene production during dietary fish oil supplementation in healthy volunteers. Submitted
41. Dayton S, Hashimoto, Dixon W, Pearce ML: Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. J Lipid Res 1966;7:103-111.
42. Kinsella JE, Lokesh B, Stone RA: Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am J Clin Nutr 1990;52:1-28.
43. Bang HO, Dyerberg J, Hjorne N: The composition of food consumed by greenland eskimos. Acta Med Scand 1976;200:69-73.
44. Picado C, Castillo JA, Schinca N, Pujades M, Ordinas A, Coronas A, Augusti-Vidal A: Effects of a fish oil enriched diet on aspirin intolerant asthmatic patients: a pilot study. Thorax 1988;43:93-97.

45. Axelrod L: Perspectives in diabetes. Omega 3 fatty acids in diabetes mellitus. Gift from the sea? Diabetes 1989;38:539-543.
46. Emeis JJ, Houwelingen AC van, Hoogen CM vd, Hornstra G. A moderate fish intake increases plasminogen activator inhibitor type-1 in human volunteers. In A.C. van Houwelingen, eds. Fish against thrombosis. Thesis 1988;158-172.
47. Virella G, Kilpatrick JM, Rugeles MT, Hyman B, Russell R. Depression of humoral responses and phagocytic functions in vivo and in vitro by fish oil and eicosapentanoic acid. Clin Immunol Immunopathol 1989;52:257-270.
48. Blonk MC, Bilo HJ, Nauta JJ, Popp-Snijders C, Mulder C, Donker AJ. Dose-response effects of fish-oil supplementation in healthy volunteers. Am J Clin Nutr 1990;52:120-127.

SUMMARY

Dietary fish oil supplementation leads to suppression of arachidonic acid derived eicosanoid synthesis in several types of cells. Since eicosanoids are actively involved in the process of inflammation this may have consequences for chronic inflammatory disorders. This thesis evaluates the clinical and laboratory effects of fish oil supplementation in patients with rheumatoid arthritis.

In the first part of *Chapter 1* a brief introduction is given to the signs, symptoms, course, prognosis, the model of tissue destruction and treatment of rheumatoid arthritis. In the second part of this chapter the metabolism of polyunsaturated fatty acids (PUFA) and the transformation of some PUFA into various cyclooxygenase and lipoxygenase products is described. In the third part the aim of this thesis is formulated.

In chapters 2, 3, 4, and 5 the effects of dietary fish oil supplementation on clinical and laboratory parameters in patients with rheumatoid arthritis are presented.

In *Chapter 2*, we describe the clinical and laboratory effects of dietary fish oil supplementation in sixteen patients. A randomized, double blind, placebo-controlled crossover design with twelve week treatment periods was used. Treatment with anti-rheumatic drugs (NSAID and DMARD) was continued throughout the study period. Placebo consisted of fractionated coconut oil flavoured with fish aroma. In comparison with placebo, a daily supply of fish oil led to significant improvements of various clinical disease variables without influencing the usual laboratory parameters of inflammation. A minor production of LTB5 was observed, concomitant with a decrease of the mean LTB4 production by in vitro stimulated neutrophils. The minor production of LTB5 was in accordance with the low amounts of eicosapentaenoic acid incorporated into the neutrophil membrane phospholipid fraction. No correlations were found between changes in the leukotriene production and the significantly altered disease variables.

Chapter 2A offers a letter to the editor regarding the persistent suppression of interleukin-1 and tumor necrosis factor produced by ex vivo stimulated mononuclear cells of healthy volunteers 10 weeks after cessation of fish oil supplementation. More studies are needed to confirm this issue, but we suggest that this 'hangover' effect is not the result of inhibition of LTB4 production.

Alpha-tocopherol is added to the fish oil capsules to prevent unwanted peroxidation of PUFA. To our knowledge little or no attention has been paid to whether the n-3 PUFA themselves, alpha-tocopherol, or the combination of both are responsible for the anti-inflammatory effects of dietary fish oil supplementation. In *Chapter 3* we compared the effects of dietary fish oil supplementation with the effects of alpha-tocopherol enriched coconut oil. Clinical disease variables were studied in relation to cellular and plasma vitamin E levels. Twenty eight patients were randomly allocated to either the fish oil or the coconut oil (placebo control) group. The follow up time was three months with clinical check ups every month. Although some clinical improvement ocurred in the control group, the improvement with fish oil was greater than that with coconut oil/alpha-tocopherol. We concluded that the n-3 PUFA, rather than the anti-oxidizing properties of the encapsuled alpha-tocopherol, are mainly responsible for the improvement of joint symptoms during fish oil supplementation in

patients with rheumatoid arthritis.

One of the mechanisms by which NSAID exert their effect is the inhibition of the production of prostaglandins whereas the production of leukotrienes remains unaffected. The ability of n-3 PUFA to affect both the cyclooxygenase and the lipoxygenase pathway should, theoretically, provide the possibility to reduce the NSAID demand in patients with rheumatoid arthritis. In *Chapter 4* the results of a double blind, placebo controlled trial to investigate whether fish oil consumption could lessen the need for indomethacin, as a model for NSAID, is discussed. The use of indomethacin fell to 81% ($p<0.05$) in the fish oil treated group and to 92% (NS) in the control group. Between group comparison revealed no statistically significant differences in lessening the need for indomethacin during fish oil and placebo treatment. The precise reason for this phenomenon is difficult to ascertain. Possibly the fish oil induced inhibition of prostaglandin production is not enough to achieve the effects as induced by a strong cyclooxygenase inhibitor like indomethacin. It is also possible that fish oil does not exert the effects of indomethacin that are independent of prostaglandin inhibition.

In most studies, fish oil preparations are provided in the semi-synthetic ethyl ester form which allows much higher concentrations of eicosapentaenoic acid than fish oil in triglyceride form. The ethyl ester form is however not natural to the human diet. Whether the induced effects of fish oil supplementation are dependent on the chemical form in which the n-3 PUFA are provided has not been well characterized yet. In *Chapter 5* we compared the efficacy of 'high' dose fish oil fatty acid ethyl ester (3.4 g eicosapentaenoic acid + docosahexaenoic acid) and 'low' dose fish oil triglyceride (1.7 g eicosapentaenoic acid + docosahexaenoic acid) preparations for altering fatty acid compositions of plasma cholesterol esters and neutrophil phospholipids, neutrophil leukotriene production and disease activity in 29 patients. We showed that a fish oil triglyceride preparation while containing half the amount of n-3 PUFA was equally effective in suppressing joint symptoms as a 'high' dose fish oil ethyl ester preparation. This was not surprising since both dosages altered the fatty acid content of plasma cholesterol esters and neutrophil phospholipids to the same extent. In addition both fish oil preparations were equally effective in inhibition of in vitro neutrophil LTB4 production and induction of LTB5 formation. Our findings indicate a diminished bioavailability of fish oil fatty acid ethyl esters. We suggest that apart from the actual amount of fish oil fatty acids present in the capsules, the chemical form in which they are provided is of importance in the induced clinical and laboratory effects in rheumatoid arthritis.

Animal studies have shown that the effect of fish oil on eicosanoid synthesis increases when combined with diets at low linoleic acid to saturated fatty acid ratios. *Chapter 6* presents the effect of low fat (25% of energy) and high fat (35% of energy) diets with either low (<0.4) or high (>1) polyunsaturated/ saturated fatty acid (P/S) ratios on fatty acid compositions of plasma cholesterol esters and neutrophil phospholipids, and leukotriene production in 4 groups of healthy volunteers supplemented daily with 6 g fish oil for 4 weeks. This study revealed that the increase in the relative amounts of eicosapentaenoic acid in plasma cholesterol esters was inversely related to the dietary intake of linoleic acid and that the eicosapentaenoic acid/arachidonic acid ratio in plasma cholesterol esters and neutrophil phospholipids

are higher in subjects consuming low P/S diets when compared with those consuming high P/S diets. Alterations in leukotriene production did not importantly differ between groups. This reflects the similar incorporation of eicosapentaenoic acid in the neutrophil phospholipid fraction of all participants. Whether neutrophil phospholipids slowly incorporate eicosapentaenoic acid or preferentially incorporate n-6 PUFA remains to be established. Further study to establish a desirable ratio of dietary n-3/n-6 PUFA to affect eicosanoid production and thereby eicosanoid mediated reactions is warranted.

In *Chapter 7* an overview is given of our work and the work of others on fish oil supplementation in rheumatoid arthritis.

CONCLUSION

In this thesis the clinical and laboratory effects of dietary fish oil supplementation in patients with rheumatoid arthritis are described. The supplement is well tolerated and provides an additive beneficial effect in suppressing joint symptoms during the use of anti-rheumatic drugs without altering the acute phase parameters of inflammation. By virtue of increasing the relative amounts of eicosapentaenoic acid in neutrophil membrane phospholipids there is a decrease in the production of proinflammatory arachidonic acid derived LTB4 while, concomitantly, small amounts of the functionally attenuated eicosapentaenoic acid derived LTB5 becomes detectable. It is however unlikely that changes in the production of leukotrienes are the only cause of clinical improvement. It should be considered that the effects of fish oil are the result of a combined inhibition of the lipoxygenase and cyclooxygenase pathway. The amount of vitamin E that is present in the capsules seems of little importance for the clinical benefit, but is necessary to prevent unwanted lipid peroxidation.

Although the relief of joint pain was appreciated by most patients it could not markedly lessen their need for NSAID. Referring to the low incorporation of eicosapentaenoic acid in neutrophil membrane phospholipids, it seems crucial to analyze the efficacy of fish oil supplementation in rheumatoid arthritis in long term clinical trials. The apparently diminished bioavailability of fish oil in its semi-synthetic ethyl ester form suggests that it is attractive to use fish oil in its natural triglyceride form in these trials. The interference of n-6 PUFA with the accumulation of eicosapentaenoic acid in cell and plasma lipids argues for further research to establish a desirable dietary n-3/n-6 PUFA ratio to optimize the influence on eicosanoid production and mediated reactions. Awaiting the results of such studies we suggest that fish oil should not be considered as a substitute for NSAID and DMARD in rheumatoid arthritis.

SAMENVATTING

Toevoeging van visolie aan het dagelijks menu, ofwel diëtaire visolie suppletie beïnvloedt de samenstelling van de celmembraan. Tijdens een ontstekingsproces worden uit vetzuren van de celmembraan de z.g.n. eicosanoïden gevormd die o.m. verantwoordelijk zijn voor pijn en de stijfheid die bij een gewrichtsontsteking optreden. Veranderingen van de vetzuursamenstelling van de celmembraan o.i.v. verandering in de vetsamenstelling van de voeding kunnen leiden tot wijziging in de produktie van deze eicosanoïden. Aangezien eicosanoïden ook betrokken zijn bij het onderhouden (=chronisch worden) van ontstekingsprocessen kan beïnvloeding van de eicosanoïd produktie gevolgen hebben voor chronische ontstekingsziekten zoals reumatoïde artritis.

Dit proefschrift beschrijft de invloed van diëtaire visolie suppletie op de klinische en biochemische maatstaven van ontstekingsactiviteit bij patiënten met reumatoïde artritis.

Hoofdstuk 1. In het eerste deel van Hoofdstuk 1 is een kort overzicht gegeven van de huidige inzichten in het ontstaan, de verschijnselen, het beloop, en de behandeling van reumatoïde artritis. In het tweede deel komt het metabolisme van meervoudig onverzadigde vetzuren en de vorming van cyclo-oxygenase en lipoxygenase produkten aan de orde. Het derde deel van Hoofdstuk 1 omvat de vraagstelling van dit proefschrift.

In de hoofdstukken 2 t/m 5 worden de effecten van diëtaire visolie suppletie bij patiënten met reumatoïde artritis beschreven.

Hoofdstuk 2. Hierin beschrijven wij de effecten van diëtaire visolie suppletie op de klinische en biochemische ernst van de ontsteking bij 16 patiënten. Het onderzoek was placebo gecontroleerd en dubbelblind opgezet met behandelingsperioden van 12 weken. Het gebruik van anti-reumatica werd gedurende het gehele onderzoek onveranderd voortgezet. Wij vonden dat visolie vergeleken met placebo, een statistisch significante en klinische relevante verbetering gaf terwijl daarentegen de uitslagen van een aantal biochemische indicatoren van ontstekingsactiviteit geen essentiële wijzigingen vertoonden. In overeenkomst met de geringe inbouw van het eicosapentaeenzuur in de celmembraan (phospholipide fractie) van neutrofiele granulocyten werd een geringe *in vitro* produktie van leukotrieen B5 meetbaar terwijl gelijktijdig de leukotrieen B4 produktie significant was afgenomen. Hoewel verandering in de produktie van leukotriënen in de tijd parallel liep met de klinische verbetering van de gewrichtsklachten bleek er geen evenredig verband te bestaan tussen de omvang van de veranderingen.

Hoofdstuk 2A wordt gevormd door de reactie op een artikel in een tijdschrift. Daarin stond vermeld dat er 10 weken na staken van diëtaire visoliesuppletie, bij gezonde vrijwilligers, nog steeds een verminderde produktie van interleukine-1 en tumor necrose factor door *ex vivo* gestimuleerde monocyten aantoonbaar was. Op grond van onze gegevens kan dit verschijnsel niet worden verklaard door een aanhoudende remming van de leukotrieen B4 produktie.

Hoofdstuk 3. Vitamine E (alpha-tocopherol) wordt standaard aan de visolie capsules toegevoegd om peroxidatie van lipiden te voorkomen. Over het algemeen is er in de literatuur weinig of geen aandacht besteed aan het feit of de visolie

(omega-3 vetzuren) zelf, het vitamine E of de combinatie van beide verantwoordelijk is voor de klinische verbeteringen. In Hoofdstuk 3 wordt een vergelijking gegeven van de effecten van diëtaire visolie suppletie enerzijds en de effecten van met alpha-tocopherol verrijkte cocosnootolie (placebo, controle groep) anderzijds, op de klinische ziektevariabelen en de vitamine E spiegels bij 28 patiënten met reumatoïde artritis. Het dubbelblinde, placebo gecontroleerde onderzoek kende een behandelingsduur van drie maanden. Alhoewel er in de controle groep ook enige klinische verbetering zichtbaar was, was de verbetering in de visolie groep meer uitgesproken. Wij concludeerden dat niet vitamine E maar de omega-3 vetzuren voornamelijk verantwoordelijk zijn voor de afneming van gewrichtsklachten van de patiënten tijdens diëtaire visolie suppletie.

Hoofdstuk 4. De uitwerking van een NSAID omvat o.a. een reductie van pijn, zwelling en stijfheid van gewrichten waarbij remming van de produktie van prostaglandines is aangetoond. Visolie kan zowel de cyclo-oxygenaseroute (prostaglandines) als de lipoxygenaseroute (leukotriënen) beïnvloeden en biedt derhalve theoretisch de mogelijkheid om het gebruik van pijnstillers te reduceren. In Hoofdstuk 4 beschrijven wij de resultaten van een placebo gecontroleerd, dubbelblind onderzoek naar de effecten van diëtaire visolie suppletie op de NSAID (indomethacine) behoefte. Na 3 maanden behandeling daalde het gebruik van indomethacine in de visolie groep doch het verschil was niet statistisch significant t.o.v. de placebo groep. Een verklaring hiervoor zou kunnen zijn dat de door visolie geïnduceerde remming van de prostaglandine produktie onvoldoende is om het NSAID gebruik te reduceren. Het is echter eveneens voorstelbaar dat indomethacine op een andere manier dan alleen via remming van de cyclo-oxygenaseroute het ontstekingsproces beïnvloedt en dat visolie niet over een dergelijke eigenschap beschikt.

Hoofdstuk 5. In veel onderzoek naar de effecten van diëtaire visolie suppletie worden de vetzuren in de semi-synthetische, ethylester vorm aangeboden. De ethylester vorm kan een aanzienlijk hogere concentratie omega-3 vetzuren bevatten dan visolie in de natuurlijke triglyceride vorm. Of de gunstige klinische effecten van visolie afhankelijk zijn van de vorm waarin de vetzuren worden aangeboden was echter tot op heden onvoldoende onderzocht. In Hoofdstuk 5 worden de effecten van een 'hoge' dosering visolie ethylesters (12 capsules = 3,4 gram omega-3 vetzuren/dag) vergeleken met de effecten van een 'lage' dosering visolie in de natuurlijke triglyceride vorm (12 capsules = 1,7 gram omega-3 vetzuren/dag). We bestudeerden de invloed op de ziekte-activiteit, vetzuursamenstelling van de celmembraan van neutrofiele granulocyten en plasma cholesterolesters terwijl eveneens *in vitro* de leukotriënen produktie van neutrofiele granulocyten werd bepaald. Het blijkt dat de 'lage' dosering visolie triglyceriden, even effectief is in het doen afnemen van gewrichtsklachten, als een 'hoge' dosering visolie ethylesters. Dit is in overeenstemming met de waarneming dat beide visolie preparaten de vetzuursamenstelling van plasma cholesterolesters en de celmembraan van neutrofiele granulocyten alsmede de *ex vivo* leukotriënen produktie in dezelfde mate bleken te veranderen. Deze resultaten suggereren een verminderde biologische beschikbaarheid van visolie vetzuur ethylesters t.o.v. visolie vetzuren in de natuurlijke triglyceride vorm. Derhalve concluderen wij dat behalve de hoeveelheid ook de vorm waarin de visolie vetzuren worden aangeboden bepalend is voor het effect.

Hoofdstuk 6. Eerder dierexperimenteel onderzoek heeft aangetoond dat de invloed van visolie op de produktie van eicosanoïden kan worden vergroot wanneer het supplement wordt gegeven in combinatie met een 'achtergrond-dieet' gekenmerkt door een lage verhouding tussen onverzadigde en verzadigde vetzuren (P/S ratio). In Hoofdstuk 6 wordt bij vier groepen gezonde vrijwilligers, tijdens diëtaire suppletie van 6 gram visolie gedurende 4 weken, de invloed bestudeerd van de vetsamenstelling van het 'achtergrond' dieet (25 vs 35 energie procenten vet; P/S<0.4 en P/S>1). Gekeken werd naar het effect op veranderingen in de vetzuursamenstelling van plasma cholesterolesters, celmembraan van neutrofiele granulocyten alsmede de *ex vivo* leukotriënen produktie. Het bleek dat de stijging van de eicosapentaeenzuur concentratie in de plasma cholesterolester fractie omgekeerd evenredig was aan de diëtaire linolzuurinname. Bovendien was bij vrijwilligers die een "achtergrond" dieet met een P/S ratio <0.4 hadden gevolgd, de eicosapentaeen/arachidonzuur ratio in de plasma cholesterolester fractie groter dan bij degenen die een P/S ratio >1.0 hadden. Dit werd -in mindere mate- ook voor de celmembraan van neutrofiele granulocyten gevonden. Het verschil in de vetsamenstelling van het dieet tijdens visolie suppletie was echter niet van invloed op de veranderingen in de leukotriënen produktie. Dit kan verklaard worden doordat de inbouw van eicosapentaeenzuur in de phospholipide fractie van neutrofiele granulocyten, tussen de groepen onderling niet significant verschilde. Of dit komt doordat de neutrofiele granulocyt de omega-3 meervoudig onverzadigde vetzuren slechts heel langzaam opneemt dan wel doordat preferentieel omega-6 meervoudig onverzadigde vetzuren worden ingebouwd is niet onderzocht.

Hoofdstuk 7. Hierin wordt een overzicht gegeven van de rol van visolie bij de behandeling van reumatoïde artritis.

CONCLUSIE

In dit proefschrift wordt de invloed van diëtaire visolie suppletie op de klinische en biochemische ontstekingsactiviteit bij patiënten met reumatoïde artritis beschreven. Het supplement heeft een gunstig effect op gewrichtsklachten terwijl het geen invloed heeft op de biochemisch gemeten ernst van de ontsteking. Visolie wordt over het algemeen goed verdragen en kan een aanvulling zijn op de gebruikelijke medicamenteuze behandeling van reumatoïde artritis. Toevoeging van visolie aan het menu leidt tot incorporatie van omega-3 vetzuren in de celmembraan van o.a. neutrofiele granulocyten. Wanneer eicosapentaeenzuur deel uit maakt van de celmembraan is er na activering van neutrofiele granulocyten *ex vivo* een verminderde leukotrieen B4 produktie aantoonbaar, terwijl tevens een produktie ontstaat van een minder krachtig pro-inflammatoir werkzame leukotrieen B5. Door het ontbreken van een duidelijke correlatie lijkt het onwaarschijnlijk dat de subjectieve klinische verbeteringen louter veroorzaakt worden door de gewijzigde produktie van leukotriënen. Het is eveneens voorstelbaar dat het klinische effect van visolie tot stand komt via een gecombineerde beïnvloeding van zowel de lipoxygenase- als de cyclo-oxygenaseroute. Vitamine E is aanwezig in de capsules om peroxidatie van de meervoudig onverzadigde vetzuren te voorkomen, maar lijkt van ondergeschikt belang voor het bereiken van het gunstige, klinische effect. Ondanks het feit dat visolie vooral pijn, zwelling en stijfheid van de

gewrichten vermindert is het na drie maanden niet mogelijk gebleken om de dosering van NSAID's te verlagen.

In het licht van de relatief geringe inbouw van het eicosapentaeenzuur in de celmembraan van neutrofiele granulocyten is verder onderzoek naar de optimale duur van de behandeling gewenst. De ogenschijnlijk verminderde biologische beschikbaarheid van visolie vetzuur ethylesters suggereert dat in een dergelijk onderzoek de visolie bij voorkeur in de natuurlijke, triglyceride vorm gebruikt dient te worden. Mogelijkerwijs leidt een reductie van de diëtaire linolzuur inname tot een toename van de inbouw van eicosapentaeenzuur in cellen en de daaraan gerelateerde veranderingen in het arachidonzuur metabolisme tijdens visoliesuppletie. Het vaststellen van een ideale diëtaire omega-3/omega-6 meervoudig onverzadigde vetzuur ratio is daarvoor evenwel noodzakelijk. In afwachting van de resultaten van bovengenoemd onderzoek mag geconcludeerd worden dat diëtaire visolie suppletie niet zozeer als vervanging maar veeleer als aanvulling op de ontstekingsremmende, anti-reumatische medicamenten gezien en gebruikt kan worden.