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Synovial fluid and plasma n3 long chain polyunsaturated fatty acids in patients with inflammatory arthritis

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ABSTRACT

Relationships between n-3 long chain polyunsaturated fatty acids (LC-PUFA) in plasma and synovial fluid (SF) were examined in 36 patients with knee effusion within the context of a variety of rheumatic diagnoses and various stated fish oil (FO) intakes (from 0 to 30 mL of standard FO daily) of variable duration. In a sub-group of patients, correlations between PUFA in SF mononuclear cells (MNC) and cell-free supernatants of SF and between SF MNC and peripheral blood (PB) MNC were examined. Correlations were also sought between clinical data (stated FO intake, pain score) and n-3 LC-PUFA.

Correlations between plasma n-3 LC-PUFA and SF n-3 LC-PUFA were very strong ($r^2 > 0.9$, $p < 0.001$). The LC-PUFA profiles of SF supernatants differed from those of MNC. PUFA profiles in PB MNC and SF MNC were similar, except for a higher proportion of DHA in the latter. Positive correlations were observed between stated intakes of FO and EPA in plasma and SF (for both $r=0.37$, $p=0.02$) and DHA in plasma ($r=0.37$, $p=0.02$) and SF ($r=0.36$, $p=0.03$). n-3 LC-PUFA in plasma and SF correlated inversely with pain score (plasma $r^2=0.16$, $p < 0.02$; SF r^2 0.32, $p=0.001$).

In conclusion, plasma n-3 LC-PUFA is a strong indicator of SF n-3 LC-PUFA status across a broad range of rheumatic diagnoses and FO intakes. Higher n-3 LC-PUFA in plasma and SF were associated with lesser pain experience.

1. Introduction

Humans are dependent on dietary intake for n-3 PUFA and also for more abundant competitor n-6 PUFA. Humans cannot synthesise n-3 alpha-linolenic acid (ALA; C18:3,n-3) and have a limited and variable capacity to convert ALA from vegetable sources to eicospentaenoic acid (EPA; C20:5n-3), n-3 docosapentenoic acid (DPA; C22:5,n-3) and docosahexaenoic acid (DHA; C22:6n-3) [1]. Direct dietary sources of EPA and DHA include fish and fish oil (FO) and to a lesser extent red meat [2]. FO is a particularly rich source of EPA and DHA and because FO has been shown to have anti-inflammatory effects, FO supplements are used medicinally to treat rheumatoid arthritis (RA) and certain other inflammatory diseases [3]. Since individuals vary in the efficiency with which they absorb ingested fat, and from time to time, in the extent to which they consume ALA and competitor n-6 PUFA [4] and in rates of conversion dietary ALA to EPA [1,5], the n-3 LC-PUFA found in blood and tissue phospholipids (PL) is not a simple reflection of dietary intakes [5].

EPA and DHA displace arachidonic acid (AA; C20:4n-6) from cell membrane PL and also inhibit synthesis of pro-inflammatory n-6 eicosanoid mediators of inflammation derived from AA [6]. EPA is also metabolised to eicosanoids, although EPA derived n-3 eicosanoids have little pro-inflammatory activity relative to their n-6 counterparts [6]. EPA and DHA are also precursors of specialized pro-resolving mediators (SPMs), which are active in the resolution of inflammation [7–9]. For these reasons, blood levels of n-3 LC-PUFA have been suggested as a potentially useful biomarker to guide medicinal use of FO [10]. However for such a biomarker to be valid, blood levels need to correlate with levels in tissues where n-3 LC-PUFA are likely to have their anti-inflammatory effects.

Multiple clinical trials have reported benefit in RA from medicinal use of FO in daily doses of 9 g or more of standard FO or a commensurate dose of a FO concentrate (for review see Refs. [3,11]). Meta-analysis of randomised controlled trials (RCTs) of FO in RA shows that FO reduces pain, tender joint count, morning stiffness and non-steroidal anti-inflammatory drug (NSAID) use in patients with RA [12]. Proudman and co-workers have more

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recently reported favourable effects of FO in recent onset RA in a long-term RCT comparing high dose FO (providing 5.5 g of EPA plus DHA daily) versus a comparator oil [13]. The latter contained a small dose of FO, providing 0.4 g EPA plus DHA daily diluted in a low n-6 vegetable oil for masking and to meet Heart Foundation recommendations for desirable EPA plus DHA intake [14]. All patients received treatment with combinations of disease modifying anti-inflammatory drugs (DMARDs), which were applied according to a predetermined treatment algorithm with rules for intensification of DMARD therapy in order to achieve predetermined treat-to-target criteria for remission or low disease activity [13]. The findings of the first 12 months of a three year study have been reported to date. Patients receiving the high dose FO required less intensive DMARD therapy and achieved more remissions than their counterparts taking the comparator blend. Furthermore, n-3 LC-PUFA as a continuous variable correlated positively with improved disease control when data from the treatment groups were pooled (Proudman SM, James MJ, Cleland LG submitted).

A role for n-3 LC-PUFA in management of chronic pain associated with inflammation is suggested by pain modulating effects in RA [12]. The possibility of a more general effect on pain has been suggested by an open study into the use of FO as an alternative to NSAIDs in patients with discogenic spinal pain [15]. At a mechanistic level, FO has been shown inhibit synthesis of the nociceptive eicosanoid prostaglandin E_2 [6,16]. Maresin-1, which is biosynthesised from DHA, has been shown to reduce pain responses in experimentally challenged mice [17]. For these reasons, possible correlations between n-3 LC-PUFA in plasma and synovial fluid (SF) and reported pain experience were examined in the present study. Correlations between stated FO dose and pain scores were also explored.

2. Materials and methods

2.1. Subjects

SF and peripheral blood (PB) samples were obtained contemporaneously from 36 patients undergoing arthrocentesis of an inflammatory knee effusion as part of their care. Mononuclear cells (MNC) were isolated from PB and SF when samples became available early enough to allow separation of MNC from other cell types within the day. Patient characteristics are shown in Table 1. All patients gave informed consent and the study protocol was approved by the Human Research Ethics Committee, Royal Adelaide Hospital.

2.2. Fish oil intake

Most patients had been told to take FO 10–15 mL daily as a complement to their routine medical management. Subjects completed a Vital Activities and Lifestyle Index (VALI) form [18] as a routine part of clinical assessment. Our modification of the VALI form includes questions on type, dose and mode of ingestion of FO taken. Answers to these questions allowed calculation of the dose of standard FO (EPA 18% w/w+DHA 12% w/w, 1 mL FO weighs 0.92 g) as mL/d. A small minority of patients took a concentrate of FO n-3 LC-PUFA as natural triglycerides, in which case the daily dose was expressed as the equivalent dose, with regard to EPA and DHA content, of standard FO in mL/day. Dose and duration of FO intake are detailed in Table 1.

2.3. Pain score

The VALI form has an uncalibrated 100 mm line for visual analogue score (VAS) for pain (0–100), which is used by patients to document intensity of pain experience.

Table 1
Demographic and clinical characteristics of patients.

Diagnosis	Age (years)	Sex	Disease duration (years)	Fish oil (mL/d)[a]	Duration of fish oil intake
Rheumatoid arthritis	82	F	30	15	6 years
Rheumatoid arthritis	83	F	18	10	8 years
Gout	64	M	30	30	8 months
Psoriatic arthritis	19	M	1	20	7 months
Crohn's arthropathy	46	F	30	11	1 year
Rheumatoid arthritis	48	F	22	15	5 years
Psoriatic arthritis	56	M	7	13	5 years
Rheumatoid arthritis	61	F	13	5	9 years
Monoarthritis	34	F	26	0	0
Rheumatoid arthritis	63	M	14	5	9 years
Gout	56	M	12	30	4 years
Rheumatoid arthritis	57	F	27	10	7 years
Gout	33	M	4	0	0
Ulcerative colitis arthropathy	50	F	1	7	1 month
Rheumatoid arthritis	60	F	38	11	9 years
Monoarthritis	62	F	1	15	2 months
Rheumatoid arthritis	62	F	2	15	1 month
Pauci-arthritis	33	F	7	10	2 years
Psoriatic arthritis[b]	58	F	6	4	5 years
MCTD[b]	55	F	3	10	3 years
Psoriatic arthritis	60	M	8	15	6 years
Rheumatoid arthritis	82	F	11	10	11 years
B27 arthropathy	62	M	41	15	1 month
Rheumatoid arthritis	66	F	10	10	1 year
Gout	54	M	10	30	2 months
Rheumatoid arthritis	68	F	29	15	3 months
Pauci-arthritis[b]	35	F	18	15	2 years
Rheumatoid arthritis	63	F	27	30	11 years
Rheumatoid arthritis[b]	42	F	8	15	3 years
Rheumatoid arthritis[b]	84	M	5	6	5 years
Monoarthritis[b]	57	M	0.1	15	1 month
Psoriatic arthritis[b]	49	M	8	0	0
CCPD[b]	67	F	1	10	1 month
B27+ spondyloarthritis[b]	53	F	30	5	12 years
Psoriatic arthritis[b]	50	M	1	15	1 year
Psoriatic arthritis[b]	49	F	12	0	0

MCTD, mixed connective tissue disease; CCPD, calcium pyrophosphate deposition disease.

[a] Dose of fish oil, whether taken as capsules or fluid on juice, standard strength or a concentrate, has been expressed as the equivalent dose of standard fish in mL/d. 1 mL standard fish oil weighs 0.92 g and contains 166 mg EPA and 100 mg DHA.

[b] Provided mononuclear cell data.

2.4. Sample preparation

PB and SF samples were collected into heparinised tubes and centrifuged at 10,000g for 15 min after which 2 mL aliquots of plasma and SF supernatant were removed and stored at −70 °C for later assay. MNC were isolated from SF and PB by density gradient centrifugation over Lymphoprep as described previously [19]. The harvested MNC were washed with phosphate buffered saline prior to drying under nitrogen. All subjects contributed samples for comparisons between plasma and SF fatty acids. When more than one sample was obtained, the initial sample for each subject was used for these comparisons. For analyses of MNC fatty acids, a subsequent SF sample was obtained in most cases. This sample was used for analyses of SF supernatants for paired comparisons with MNC. Comparisons between SF MNC and PB MNC were undertaken using contemporaneously obtained samples.

2.5. Fatty acid analysis

Plasma, SF supernatants and MNC preparations were treated with chloroform/isopropanol to extract lipids. The PL fraction was separated by thin layer chromatography and subjected to