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Randomized control trials

Docosahexaenoic acid in the treatment of rheumatoid arthritis: A double-blind, placebo-controlled, randomized cross-over study with microalgae *vs.* sunflower oil

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SUMMARY

The potential of fish or fish oil as supplier for eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) for reducing cardiovascular risk factors and supporting therapy of chronic inflammatory diseases, has been investigated intensively, but our knowledge about the physiological effects of the individual compounds EPA and DHA are limited.

Study design: In this double-blind pilot study, thirty-eight patients with defined RA were allocated to consume foods enriched with microalgae oil from *Schizochytrium* sp. (2.1 g DHA/d) or sunflower oil (placebo) for 10 weeks (cross-over), maintaining the regular RA medication during the study.

Results: In contrast to placebo, the daily consumption of DHA led to a decline in the sum of tender and swollen joints (68/66) from 13.9 ± 7.4 to 9.9 ± 7.0 ($p = 0.010$), total DAS28 from 4.3 ± 1.0 to 3.9 ± 1.2 ($p = 0.072$), and ultrasound score (US-7) from 15.1 ± 9.5 to 12.4 ± 7.0 ($p = 0.160$).

The consumption of placebo products caused an increase of the n-6 PUFA linoleic acid and arachidonic acid (AA) in erythrocyte lipids (EL, $p < 0.05$). The amount of DHA was doubled in EL of DHA-supplemented patients and the ratios of AA/EPA and AA/DHA dropped significantly. We speculate that the production of pro-inflammatory/non-resolving AA-derived eicosanoids might decrease in relation to anti-inflammatory/pro-resolving DHA- and EPA-derived lipid mediators. In fact, plasma concentrations of AA-derived thromboxane B_2 and the capacity of blood to convert AA to the pro-inflammatory 5-lipoxygenase product 5-hydroxyeicosatetraenoic acid were significantly reduced, while levels of the DHA-derived maresin/resolvin precursors 14-/17-hydroxydocosahexaenoic acid significantly increased due to DHA supplementation.

Conclusion: The study shows for the first time that supplemented microalgae DHA ameliorates disease activity in patients with RA along with a shift in the balance of AA- and DHA-derived lipid mediators towards an anti-inflammatory/pro-resolving state.

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; COX, cyclooxygenase; CRP, c-reactive protein; DAS28, disease activity score; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DMARDs, disease-modifying antirheumatic drugs; DPA, docosapentaenoic acid; EL, erythrocyte lipids; EPA, eicosapentaenoic acid; ESR, Westgren erythrocyte sedimentation rate; FA, fatty acid; FAME, fatty acid methyl esters; FFP, Food Frequency Protocol; GLA, γ-linolenic acid; HAQ, health questionnaire; 17*S*-HDHA, 17*S*-hydroxy-docosahexaenoic acid; HETE, hydroxy-eicosatetraenoic acid; LA, linoleic acid; 5-LOX, 5-lipoxygenase; LPS, lipopolysaccharide; LPS/fMLP, lipopolysaccharide/N-formyl methionyl-leucyl-phenylalanine; LT, leukotrienes; MUFA, monounsaturated fatty acids; n-3 LC-PUFA, n-3 long-chain polyunsaturated fatty acids; NSAIDs, nonsteroidal anti-inflammatory drugs; PG, prostaglandins; PL, plasma lipids; RA, rheumatoid arthritis; RBC, red blood cells; RRs, relative ratios; SFA, saturated fatty acids; TBX, thromboxanes; UPLC MS/MS, ultraperformance liquid chromatography-coupled ESI tandem mass spectrometry; US-7, ultrasound score-7.

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1. Background

Epidemiological studies as well as clinical investigations indicate a beneficial impact of long-chain n-3 polyunsaturated fatty acids (n-3 LC-PUFA), such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) on the incidence as well as on the clinical and immunological parameters in patients with rheumatoid arthritis (RA; [1–19])

Previous human trials suggest that dosages of about 3 g/d of n-3 LC-PUFA for at least 12 weeks are sufficient to reach clinical benefits in RA patients [20], whereas studies with lower dosages of n-3 LC-PUFA failed to show significant clinical benefits or anti-inflammatory effects [4,10,19,21]. Similar to n-3 LC-PUFA supplementation the consumption of a diet low in arachidonic acid (AA; <50 mg/d AA) reduces clinical signs of inflammation in RA patients suggesting a synergism between low AA intake and high intake of EPA and/or DHA.

The consumed supplements used in the previous studies generally contained EPA and DHA in a ratio of 2–3:1, which is characteristic for fish oil. Considering the limited availability of fisheries resources there is high demand for alternative sources of n-3 LC-PUFA. In this respect, the microalgae *Schizochytrium* sp. as alternative to fish oil is promising due to its DHA-rich oil.

In our pilot study presented here, we investigated the clinical benefit of daily intake of foods enriched with microalgae oil as source of DHA in RA patients. In particular, the influence on disease activity and changes in the profile of pro-inflammatory/non-resolving and anti-inflammatory/pro-resolving lipid mediators was examined.

2. Methods

2.1. Subjects

Thirty eight RA patients (32 f, 6 m) living in Jena (Germany), diagnosed according to the 2010 revised criteria of the American Rheumatism Association, were included in the study after giving their written informed consent.

Patients with at least moderate disease activity, as defined by DAS28 $\geq$ 2.4 were included. The intake of either nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids equivalents ($\leq$10 mg/d prednisone) or disease-modifying anti-rheumatic drugs (DMARDs) were eligible. The dosage of NSAIDs had been stable for at least 2 weeks before day 1 of the study and daily intake of corticosteroids and DMARDs had to be on a constant dosage for at least 4 weeks before and remained below this limit throughout the study (30 weeks). Subjects diagnosed with gastrointestinal or metabolic diseases, regularly alcohol abuse, dietary supplement intake (e.g., fish oil capsules), and with known food allergy or intolerance were excluded.

Patients were withdrawn from the study at any time after enrollment for the following reasons: patient's request, serious infection, inadequate control of arthritic symptoms ($\geq$50% increase in the number of swollen or tender joints), intra-articular corticosteroids within 4 weeks prior to randomization or blood sampling, reinstitution of DMARD therapy, additional prednisone need (>additional 10 mg/d prednisone equivalent), or if patient compliance with the study protocol was equivocal.

The study was conducted in accordance the Helsinki Declaration of 1975 as revised in 1983. The study protocol was approved by the Ethical Committee of the Friedrich Schiller University of Jena (reference number 2609-07/09) and registered by ClinicalTrials.gov (NCT01742468).

2.2. Study design and clinical evaluation

Study recruitment started in December 2012. At enrollment, the patients were randomly divided into verum or placebo groups (allocation ratio 1:1; Fig. 1). The placebo-controlled, randomized double-blinded cross-over study consisted of two 10-week intervention periods with a 10-week washout period in between (Fig. 2). Venous blood was collected and disease activity was assessed before and after each period. The sum of the number of swollen joints (total 66) and the number of tender joints (total 68) as well as disease activity score DAS28 (composed of the number of swollen joints (total 28), the number of tender joints (total 28), Westgren erythrocyte sedimentation rate (ESR), and patients global disease assessment) were determined. Ultrasound examinations were performed at one hand and one foot (total 7 joints). Soft tissue changes (synovitis and tenosynovitis) and erosive bone lesions in seven preselected joints were analysed using the German US-7 score [22]. C-reactive protein (CRP), health assessment questionnaires (HAQ), and duration of morning stiffness were also determined.

Subjects documented their nutritional habits in a *Food Frequency Protocol* (FFP) over the last week (7 days) of period I (including daily consumption of study products) and in the week before commencement of period II (without consumption of study products; Fig. 2). The FFP originated from Prodi® 5.4 software (Nutri-Science, Freiburg, Germany). Additionally, the daily consumption (type and amount) of meat, sausage, fish, fats and oils, as well as daily pain evaluation (0, no pain–4, very strong pain) and daily medication use were documented in the study diary (Fig. 2). Analyses of fatty acid compositions of commercial foodstuffs carried out in our lab (unpublished data) and provided by nutrition value tables [23] were used for calculating fatty acid intake during the normal diet.

2.3. Intervention/study diet

Intervention products (60 g sausage, 8 g tomato spread and 30 g milk powder (= 200 mL milk beverage) were enriched with 8 g microalgae oil (*Schizochytrium* sp., Maris DHA oil, no. 3790, IOI, Hamburg, Germany) resulting in a DHA dose of 2.11 g/d (Table S1). Placebo products (60 g sausage, 8 g tomato spread and 30 g milk powder) were enriched with 8 g commercial available sunflower oil (PPM, Magdeburg, Germany) rich in oleic acid (18:1 n-9, OA; 28%) and linoleic acid (18:2 n-6, LA; 61%, Table S1). The total fat content of both product groups was comparable and products were offered in neutral packaging.

2.4. Analytical procedures

A detailed protocol is available as supplemental data. In brief, blood collection, preparation of red blood cell fraction (RBC), lipid extraction and fatty acid (FA) analysis was carried out as described in Dittrich et al. [24]. The analysis of lipoxygenase (LO) and cyclooxygenase (COX) metabolites in human plasma was conducted by HPLC [25–27] as well as by ultraperformance liquid chromatography (UPLC)–coupled ESI tandem mass spectrometry (UPLC–MS/MS) [27,28]. LTB_4 and PGE_2 levels of unstimulated blood were determined in plasma with commercially available kits (LTB_4: Sapphire Bioscience, Waterloo, Australia; PGE_2: Biotrend, Köln, Germany).

2.5. Statistical analysis

Results are shown as mean ± SD. Changes on the sum of tender joints and swollen joints (68/66), disease activity score DAS28 and US-7 score after ten weeks of treatment were define as primary outcome measures.

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