A randomized double blind comparison of short-term duodenally administrated whale and seal blubber oils in patients with inflammatory bowel disease and joint pain

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1. Introduction

Inflammatory bowel disease (IBD) comprises Crohn's disease (CD) and ulcerative colitis (UC), both chronic inflammatory diseases of the intestinal mucosa of unknown aetiology. Rheumatic complications are common, particularly joint pain with or without clinical signs of arthritis [1]. Joint pain may have considerable negative impact on the patients' health related quality of life (HRQOL) [2]. Pro-inflammatory eicosanoids derived from arachidonic acid (20:4n-6, AA), particularly nociceptive prostaglandin E2 (PGE_2), prevail in blood and tissues of people in the Western world due to an imbalance in the n-6 to n-3 fatty acid ratio of their diet [3]. Non-steroidal anti-inflammatory drugs (NSAIDs) are non-selective inhibitors of cyclooxygenase (COX) which rapidly relieve pain by targeting prostaglandins like PGE_2 [4]. However the use of NSAIDs is hampered by gastrointestinal complications and possibly even deterioration of IBD [5]. Besides, the use of new selective COX-2 inhibitors is associated with increased risk of cardiovascular events [4]. In an effort to cope with pain and discomfort of daily life, many patients seek complementary or alternative medications (e.g. dietary supplements) [6].

The initial studies on the long chain (LC) n-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) were performed in Greenland Eskimos [7]. This particular population ate more blubber and meat from seals and whales than they ate fish products [8] and had low prevalence of chronic inflammatory diseases like rheumatoid arthritis (RA) [9, 10]. Compared with fish oil, seal blubber oil (SO) and particularly whale blubber oil (WO) contains less EPA and DHA but more of docosapentaenoic acid (22:5n-3, DPA), a potent inhibitor of platelet aggregation [11-14]. SO and WO are structurally different oils compared with fish oil. EPA and DHA are almost exclusively located in the *sn*-1 or *sn*-3 position of triacylglycerol (TAG) from WO and SO, while these fatty acids are located mainly in *sn*-2 position of TAG from fish oil [15]. *Sn*-

1/*sn-3* position specific lipoprotein lipases [16] may possibly favour peripheral availability of LC n-3 PUFA in outer positions of TAG, as seen with PGE_2 reduction in rats fed structured TAG [17]. Whether this also applies for natural TAGs with complex fatty acid compositions remains unknown.

Fish oil supplementation partially replaces AA with EPA and DHA in cell membranes, where EPA competes with AA for being substrate for the production of eicosanoids, thus inhibiting the generation of pro-inflammatory eicosanoids like PGE_2, similar to NSAIDs, and in addition EPA may be substrate for anti-inflammatory eicosanoids [3]. Nociceptive PGE_2 is a key pro-inflammatory modulator [3], and inhibition of this biomarker can be utilised to predict drug effects [18]. While clinical evaluation of pain remains subjective, it should not be ignored [6]. Visual analogue scale (VAS) scores together with HRQOL are widely used in this context. In RA patients, oral intake of fish oil is a safe remedy reducing joint pain intensity and need for NSAIDs after a latency of 2 to 3 months [12, 19]. However, when administered intraduodenally, the ameliorating effect of marine oils may appear much sooner. In an open pilot study, duodenal administration of SO (10 mL × 3/day for 10 days) ameliorated IBD-related joint pain and IBD-disease activity [20]. The effect was reproduced in a subsequent randomized controlled study where the beneficial effect of SO on joint pain and HRQOL was significant compared with soy oil [21, 22]. In fact, while SO had prolonged effect on joint pain, soy oil tended to worsen the condition, indicating that relief of joint pain is a specific effect of the type of oil given. The studies also suggest that the route of administration may significantly influence the effect.

Health effects of administrating WO have been scarcely investigated. In one previous study, WO was shown to inhibit tumor necrosis factor-α (TNF-α) more effectively than fish oil in healthy volunteers [13, 23]. Whether WO has pain modulating effects has not been examined previously. The present pilot study aimed at comparing the effects of SO (active

control group) and WO (experimental group) on IBD-related joint pain after short-term duodenal administration.

2. Patients and Methods

Study design and patients

Out-patients at Haukeland University Hospital (Bergen, Norway) between 18-75 years old with IBD, i.e. CD or UC as assessed by a gastroenterologist, in combination with presence of joint pain, with or without diagnosed arthritis (i.e. arthralgia) as assessed by a rheumatologist, were eligible for the study. Patients with highly active IBD, requiring more than 10 mg of oral steroids/day, were excluded, as well as lactating mothers and those who planned or had confirmed pregnancy. Changes in dosage or type of disease modifying anti-rheumatic drugs (DMARD), drugs that reduce bowel inflammation, or receiving intraarticular glucocorticoids, all within four weeks before inclusion and during study, were also reasons for exclusion. NSAIDs and simple analgesics were allowed to be used on demand. The study was approved by the Regional Committee for Medical Research Ethics and carried out in accordance with the Helsinki Declaration. Before inclusion, patients gave written informed consent.

During the period November 2004 to September 2005, twenty-two out-patients were assessed for eligibility of whom 19 patients were included (two had osteoarthritis and one had hyper-mobile joints in combination with highly elastic tissue and were excluded). Patients were randomised to the two treatments in blocks of two. One patient in the WO group dropped out early due to problems with the feeding tube. The remaining nine patients in each group received WO or SO supplementation in a double blind manner (Table 1). A nurse not involved in the treatment or analysis of data administered the allocation to treatment, and the randomization codes were not revealed to the investigator analyzing data until completion of data analysis. The patients were instructed to abstain from LC n-3 PUFA intake (fish, seafood and dietary supplements) for a seven-day run-in period and during the 10-day study period. Otherwise, they were instructed to maintain their normal Western diet.

In the morning, after an overnight fast, a nasoduodenal feeding tube (Freka® Feeding Tube, Fresenius Kabi, GmbH, Germany) was inserted by aid of fluoroscopy until its tip was located in the distal part of the duodenum. Ten mL of SO (*Arctic Omega-3 seal oil,* purchased from JFM Sunile AS, Os, Norway) or WO (donated by Myklebust Trading AS, Myklebost, Norway) were self-administered through the feeding tube for 10 days, three times daily before meals. As tube feeding is not feasible for long-term treatment, a 10-day study period was chosen as a compromise. After feeding, the tube was flushed with 10 mL lukewarm tap water. At bedtime, the tube was flushed with 20-50 mL of slightly hot tap water to prevent clogging of the tube.

Experimental oils

The WO is a molecularly distilled (thin film, short path facility) oil from adult minke whales (*Balaenoptera acutorostrata*) and the SO is refined oil from adult harp seals (*Phagophilus groenlandicus*). Both oils are approved according to current legislations on contaminants. The fatty acid composition analysed by gas liquid chromatography (GLC), the levels of fat soluble vitamins A, D and E analysed by high performance liquid chromatography (HPLC) and the lipid peroxidation analysed by thiobarbituric acid reactive substances (TBARS) in the WO and SO, are presented in Table 2. The commercial SO contained a mixture of natural antioxidants (α, β, γ and δ tocopherol) from soy beans. The non-commercial WO was added 1000 ppm of synthetic dl-α-tocopherol (Roche Vitamins Ltd, Basel, Switzerland), but the mixing of the sticky antioxidant into WO proved difficult, and the level of α-tocopherol in WO was low (Table 2). Oils were protected with nitrogen on top in bottles, and stored in refrigerator during study, otherwise in -20°C freezer. Thirty mL oil, as in all our previous studies [20-22, 24, 25], provided a daily dosage of 3.1 and 4.3 g EPA+DHA and 3.8 and 5.4 g

marine n-3 PUFA (EPA+DPA+DHA) in WO and SO groups respectively, which is above anti-inflammatory dosages (min 2.7 g EPA+DHA/day) used in RA studies [26].

Measurements in the morning before and after 10 day's oil treatment

Duration of morning stiffness referring to the last week in min (maximum 720 min) was estimated by the patients. Numbers of painful joints were counted with a reduced 28 joint count [27], with the addition of ankle joints and toes, toes scored as one, i.e. totally 40 joints. The intensity of joint pain, intensity of back pain, and total influence of pain, all referring to the last week, were evaluated separately by means of a 100 mm horizontal VAS ranging from 0 (very well) to 100 (very poor) [28].

IBD-disease activity was evaluated by Harvey-Bradshaw simple index in CD [29] and Walmsley simple clinical colitis activity index in UC [30]. These two IBD-indexes consist of four clinical criteria: symptoms; physical signs; general well-being and extraintestinal complications. Scores for the two diseases were pooled as in previous studies [20, 22, 24]. A score equal to or higher than 6 indicates active IBD. In addition, IBD-disease activity was evaluated by the non-invasive biomarker faecal calprotectin [31]. Patients collected spot samples of faeces, which were stored at -20° C for subsequent analysis of calprotectin by enzyme-linked immunosorbent assay (ELISA) kit for determination of calprotectin in stools (CALPRO AS, Oslo, Norway). Lower and upper limits of the standard curve were 20 and 1275 mg/kg faecal calprotectin respectively. Faecal calprotectin levels exceeding 500 mg/kg indicate active IBD [31].

Quality of life (QoL) was assessed by the short form of the Nepean Dyspepsia Index (SF-NDI), originally developed for functional dyspepsia. We used a Norwegian translated version, validated in patients with subjective food hypersensitivity [32]. SF-NDI consists of total score and 5 subscores: tension; interference with daily activities; eating/drinking;

knowledge/control and work/study. Each subscore has a range of 2-10, giving a range of total score from 10-50, where higher scores indicate worse functioning or symptoms.

Adverse effects were determined by a simple self-constructed questionnaire about abdominal gas/bloating, stomach pain/discomfort, loose stools/diarrhoea, nausea and regurgitation during the last week. All symptoms were graded from 1-4 (1 = not at all, 2 = to some extent, 3 = to a large extent, 4 = all the time) and a total score (5-20) was calculated, where higher scores indicate worse symptoms.

The WO and SO are comparable regarding taste, smell and appearance. At commencement of study, the patients were asked which oil they would prefer (WO, SO or no preference). At end of study, they were questioned which oil they thought they had received (WO, SO, or no clue). Habitual seafood intake was reported descriptively in brief.

Venous plasma samples from fasting patients were collected in vials with K_2-EDTA anticoagulant, centrifuged and stored at -80° C prior to analysis of fatty acid composition and PGE_2. After centrifugation, before storage at -80° C, plasma for PGE_2 analysis was added the COX-inhibitor indomethacin (Sigma-Aldrich, Saint Louis, USA); dissolved in ethanol, final concentration of 10 µg indomethacin/mL plasma, within 30 minutes of venupuncture. Duplicate aliquots of plasma without indomethacin were taken for control.

Fatty acid composition of total lipids in plasma and marine oils was analysed by GLC according to previously described methods [20, 33], using biological sample parallels, except every 10^{th} sample in duplicate. Total lipid content was extracted, filtered and evaporated, sample saponified and fatty acids were esterified. The methyl esters were separated using Auto-GC (Instrument-Teknikk AS, Norway), equipped with a 50 m CP sil 88 (Chrompack) fused silica capillary column (id: 0.32 mm), using ”cold on column” injection, temperature programme $60^{25°C/min}160^{25°C/min}190^{25°C/min}220°C^{5min}$, and flame ionization detector. The fatty acid composition was calculated using an integrator (Turbochrom Navigator, Version 6.1),

connected to the GLC and identification ascertained by standard mixtures of methyl esters (Nu-Chek, Elyian, USA). Nonadecanoic acid (19:0) methyl ester was used as internal standard. Limit of quantification (LOQ) was 10 μg fatty acid/g sample (wet weight, w/w).

PGE_2 was extracted from plasma with and without added indomethacin and analysed using liquid chromatography tandem mass spectrometry (LC-MS^n) according to previously described methods [34, 35]. The method involves precipitation of the protein fraction, centrifugation, evaporation and dissolution of the supernatant in acetonitrile and quantification by liquid chromatography ion trap mass spectrometry (LCITMS). The LCITMS used was an Agilent 1100 series LC/MSD trap, SL model with an electrospray interface (ESI). A Zorbax Eclipse-C_8 RP 150 × 4.6 mm, 5 μm (Agilent Technologies. Palo Alto, CA, USA) column held at 40 °C with acetonitrile isocratic mobile phase at 0.2 mL/min and 25 μl injection volumes were used. The ESI source was operated in negative ion mode, isolating and fragmenting the *m/z* 351 → 333, 315, 271 for PGE_2. Software used was ChemStation for LC/MSD version 4.2 from Agilent. PGE_2-*d4*, deuterated analogue of PGE_2, was used as internal standard. LOQ was 0.4 ng PGE_2/ml.

Vitamin A, i.e. sum retinol (13-, 11-, 9-cis and all-trans retinol, i.e. A_1) and 3,4 didehydro-all-trans retinol (A_2) in marine oils was analysed by a modified HPLC method [36, 37]. Briefly, the sample is saponified, while the unsaponified sample material is extracted, and analysed by a HPLC column (HICHROM 4,6 × 150 mm, LC-SI, 3μm, Teknolab A/S) using UV-detector (Thermo Separations products, UV1000, Instrument-Teknikk AS) with reference to an external standard curve. LOQ in oils was 280 ng vitamin A_1/g sample and 460ng vitamin A_2/g sample, both (w/w).

Vitamin D in marine oils was analysed by HPLC as previously described [38]. In brief, sample material is saponified and the unsaponified material is extracted before clean-up on a preparative column (HICHROM, Kromasil silica, 5 μm, 4.6x250 mm). The fraction with

vitamin D_2 and D_3 is collected (normal phase), evaporated and dissolved in methanol, before injected on an analytical column (Ace 5 C18, 5 μm, 4.6 × 250 mm), (reverse phase). Vitamin D_3, in our case, was determined by UV-detector (LaChrom, Merck HITACHI L-7420). Vitamin D_3 was quantified using vitamin D_2 as internal standard. LOQ was 1 μg vitamin D_3/ 100 g sample (w/w).

Vitamin E in marine oils was analysed by HPLC, based on the principles reported by European Committee for Standardization (CEN) [39]. Briefly, sample is saponified and unsaponified sample material is extracted. α-, β-, γ- and δ-tocopherol isomers were determined using a HPLC column (LiChroCART, 4,6 × 125 mm, Purospher STAR Si, 3 μm, Merck) equipped with a fluorescence detector (TSP, FL3000, Spectra system) and quantified by reference to an external standard curve. In our case, only α-tocopherol was reported. LOQ in oils was 500 ng α-tocopherol/g sample (w/w).

TBARS was determined in marine oils by a modified in vitro method, measuring mainly malondialdehyde and other aldehydes, secondary lipid oxidation parameters [40, 41]. In brief, fat and water-soluble components are separated, while the analyte is extracted in methanol:water phase. An aliquot of the latter phase is added thiobarbituric acid (TBA) in excess and heated to form a coloured complex between aldehydes in the sample and TBA. The absorption at 532 nm was registered and TBARS was quantified by reference to an external standard curve in a spectrophotometer (Shimadzu recording spectrophotometer, UV 240). LOQ was 3.9 nmol TBARS/g sample (w/w).

Statistics

Values were expressed as mean ± standard error of the mean (SEM) for normally distributed data, otherwise median and range (the latter reported in brackets) were used. Data normality was tested with Shapiro-Wilk test. Group differences were evaluated by unpaired Student's t-

test or Mann Whitney test (two-sided). Effect of treatment was calculated as change (in absolute values) from baseline. Paired differences were evaluated by paired t-test or Wilcoxon sign rank test. *P* values < 0.05 were regarded statistically significant. Data were analysed and displayed using the GraphPad Prism 4 (GraphPad Software Inc, San Diego, USA) statistical software package. Analytical measurements between limit of detection (LOD) and LOQ were set at LOQ, and levels below LOD regarded as not detected.

3. Results

Baseline levels

In the present study the effect of treatment appeared similar in patients with CD and UC, in patients with and without arthritis, and in men and women. Patient measures for these groups were therefore pooled. The two treatment groups were comparable regarding baseline characteristics, except a tendency of more NSAID use in the WO group, consistent with the higher number of patients with arthritis (Table 1) and a higher score on total influence of pain last week in this group ($P = 0.03$, Table 3). At inclusion, the WO group also tended to have higher IBD-disease activity (6 and 3 patients in WO and SO groups respectively with IBD-index of at least 6 indicating active IBD). Faecal calprotectin levels indicated nonactive IBD in all but two patients in each group (Table 3). Due to these minor inequalities at baseline, the effects of treatment were displayed as change in absolute values from baseline. Most patients reported that fish, seafood or LC n-3 PUFA supplements were part of their regular diet, with a slightly higher consumption in the SO group.

Fatty acid profile

Levels of the individual LC n-3 PUFAs EPA, DPA and DHA as well as sum LC n-3 PUFA increased in plasma, while AA to EPA ratio and n-6 to n-3 fatty acid ratio decreased in plasma after both treatments (Table 4), without any significant group differences. DHA tended to increase more in the WO group ($P = 0.06$). While sum n-6 fatty acids and linoleic acid were significantly reduced after both treatments, the changes were significant for SO group when compared with WO group ($P = 0.02$) (Table 4). Dihomo γ-linolenic acid (DGLA, 20:3n-6) was significantly reduced in both groups (Table 4). Sum total fatty acids, sum saturated fatty acids, alpha-linolenic acid (18:3n-3), tetradecanoic acid (14:0) and hexadecanoic acid (16:0) were significantly reduced in SO group compared with WO group

($P = 0.002$, $P = 0.009$, $P = 0.007$, $P = 0.02$ and $P = 0.007$ respectively, Table 4). Sum monounsaturated fatty acids was reduced in SO group ($P = 0.001$, Table 4). Level of AA tended to reduce after SO treatment ($P = 0.06$, Table 4). Stearic acid (18:0) was significantly reduced in SO group, with a tendency in WO group ($P = 0.07$, Table 4). Oleic acid (18:1n-9) was significantly reduced in SO group ($P = 0.0003$, Table 4).

Prostaglandin E2

PGE_2 level in plasma added indomethacin was significantly reduced from 1.27 ng/ml (0.012-2.08 ng/ml) to 0.63 ng/ml (0.012-2.41 ng/ml) after SO treatment ($P = 0.02$). Although the WO group revealed a reduction from 0.84 ng/ml (0.27-3.06 ng/ml) to 0.34 ng/ml (0.012-2.13 ng/ml), the reduction in this particular case was not significant ($P = 0.07$). However, the difference in change between groups was not significant ($P = 0.93$) (Figure 1). PGE_2 level in plasma without indomethacin did not show such consistent reductions during treatments (data not shown).

Joint pain measures

Compared with baseline, scores on VAS for intensity of joint pain, intensity of back pain and total influence of pain (all last week) were significantly reduced after both WO ($P = 0.02$, $P = 0.03$ and $P = 0.02$ respectively) and SO administration ($P = 0.002$, $P = 0.02$ and $P = 0.008$ respectively), with no significant group differences (Table 1, Figures 2-4). There was a tendency of reduced number of painful joints after WO treatment ($P = 0.07$), while duration of morning stiffness was not significantly altered by WO or SO treatment (both $P = 0.13$).

IBD-disease activity

Indexes of IBD-disease activity were significantly reduced after administration of both WO ($P = 0.02$, to a level below six indicating non-active IBD) and SO ($P = 0.049$), without any significant group difference (Table 3). Levels of faecal calprotectin remained low indicating non-active IBD (Table 3). None of the patients had faecal calprotectin levels indicating active IBD after treatment (Table 3).

Quality of life

SF-NDI total score decreased from 24 (11-37) to 17 (12-31) with WO treatment ($P = 0.04$) and from 18 (11-29) to 14 (11-30) with SO treatment ($P = 0.03$). Daily activity interference subscore decreased from 4 (2-6) to 3 (2-5) with SO treatment ($P = 0.03$). There were no significant group differences for any of these measures. There were also tendencies of reduction in knowledge/control subscore ($P_{WO} = 0.06$), tension subscore ($P_{WO} = 0.08$) and work/study subscore ($P_{SO} = 0.06$).

Adverse effects

No significant adverse symptomatic effects of oil supplementation were observed. Regarding nausea, two patients in WO group experienced increased nausea and three patients in SO group experienced decreased nausea during treatment, i.e. there was a tendency of less nausea after SO compared with WO administration ($P = 0.09$ between groups).

Preferences and Blinding

Nine patients (50 %) had preference for one of the oils (all for SO) before treatment (WO/SO groups: four/five), but such preference did not predict a better outcome than for those nine patients (50 %) who did not have any preference at baseline. After treatment, 11 patients did

not know which oil they had been taking (WO/SO groups: five/six), two answers were missing from WO group and five patients thought they had taken WO, however only two were correct. Thus at least 14 patients (78 %) were unaware of which oil they received.

4.Discussion and Conclusion

After 10-days' duodenal administration of WO or SO, the following significant changes were seen: reduced plasma AA to EPA ratio and PGE_2 levels (tendency in WO group), decreased IBD-related joint pain and IBD-disease activity, and improved QoL, with no significant group differences. The results are consistent with our prior finding of marked relief of joint pain after duodenal administration of SO [20-22], but as long as WO appeared similarly effective as SO, symptomatic effects of the oils remain unproved in the present study. Consistent with previous studies on SO administration in patients without highly active IBD [20, 22, 24], no adverse effects were observed.

The authors acknowledge that oral administration of marine oils for the relief of joint pain is a long-term strategy [12]. However, many IBD-patients with joint pain clearly need safe analgesics on demand. Although no comparative study of duodenal versus oral administration of marine oils for relief of joint pain exists, orally administered SO had no significant effect after 14-days' administration in randomized controlled trials with patients suffering IBD or psoriatic arthritis [24, 42]. The results thus suggest that duodenal administration is important for rapid effect. Duodenal administration may stimulate vago-vagal anti-inflammatory reflexes, inhibiting the release of pro-inflammatory cytokines like TNF-α and interleukin 6 [43]. A large bolus of duodenally administered marine oils may also challenge the digestive and absorptive capacity of the intestine, possibly thereby influencing both gut microbiota [44] and mucosal defence. Interestingly, retarded release phosphatidyl-choline administration appears effective in the treatment of UC [45] and enteric coated capsules with LC n-3 PUFA designed for distal delivery may have prophylactic effect in CD [46]. Hence, there are several possibilities of improved effect by duodenal administration of oils from marine mammals.

Previously, short-term duodenal administration of SO reduced blood and rectal mucosa n-6 to n-3 fatty acid ratio and AA to EPA ratio [20, 21]. While SO induced significant reductions in plasma n-6 PUFA and LA content compared with WO in the present study, both WO and SO administration reduced plasma n-6 to n-3 fatty acid ratio and AA to EPA ratio (Table 4). The AA to EPA ratio is rate limiting for eicosanoid production [3]. In IBD-patients, short-term (14-day) oral administration of SO and cod liver oil reduced plasma levels of leukotriene B4 (LTB_4) [24], and decreased levels of PGE_2 in colonic mucosa [47] and blood mononuclear cells [48] have been seen after long-term oral fish oil supplementation. Indeed reduced synthesis of nociceptive PGE_2 due to COX-inhibition is a generally accepted mechanism by which fish oil as well as NSAIDs/COX-2 inhibitors ameliorate inflammatory pain [26, 4]. In a previous study, a combination of SO and cod liver oil reduced PGE_2 levels [15]. The present study indicates for the first time reduced plasma PGE_2 levels after SO administration alone, suggesting that oils from marine mammals may also relieve joint pain by inhibition of COX. As α-tocopherol reduces the release of AA from phospholipids [49], the 30 times lower α-tocopherol level in WO, together with low EPA content, may possibly explain an insignificant effect of WO on PGE_2 level. While the positional distribution of LC n-3 PUFA on TAG in SO and WO is potentially beneficial [17], it remains unknown whether it has a bearing on PGE_2 reduction as no additional fish oil group was included.

Besides somewhat different EPA and DHA levels, SO and WO also contained 3.7 and 2.2 g/100 g DPA respectively. Plasma phospholipid DPA was recently found to correlate inversely with C-reactive protein in patients with active IBD [50]. SO and WO also contained 1.3 and 2.3 g/100 g stearidonic acid (18:4n-3) respectively, possibly weighing partially up for the low EPA content compared with fish oil [51]. While no significant group difference compared with SO, 14-days' oral administration of cod liver oil, rich in LC n-3 PUFA, vitamin D (60 μg/30 mL, i.e. 874 % of recommended daily allowance, RDA) and vitamin E,

previously induced with-in group reduction of IBD-related joint pain [24]. Interestingly, mice lacking vitamin D receptor develop experimental colitis, and it has been suggested that vitamin D deficiency may increase the risk of IBD [52] and that vitamin D supplementation may be beneficial in CD patients [53]. In the present study, WO or SO provided 4.3 μg or 0.8 μg vitamin D/day, 57 or 11 % of RDA respectively. Thus, EPA and DHA may not alone be responsible for the effects of WO and SO in IBD-patients with joint pain.

Study limitations are lack of placebo group, a small material, a minor baseline group difference and concomitant use of NSAIDs, which are difficult to withdraw. Placebo effects are inherent in clinical interventions, particularly when assessing symptoms. Including a placebo group would be ideal, however ethical and practical considerations prohibited such a study design in the present pilot study with limited number of patients and difficulty in finding an appropriate placebo to be given duodenally as soy oil may be deleterious [54, 22]. As suggested in the Helsinki Declaration we therefore chose SO as active control [21, 22]. The fact that none of the patients had preference for WO while 50 % of the patients had preference for SO may reflect scarce knowledge and availability of WO. Also, few patients were able to correctly identify which oil they had been taking, and the oil preferences did not predict outcome. Hence, response expectations had probably minor influence on the relative effects of SO and WO.

In conclusion, when administered duodenally for 10 days', no significant group differences were observed between WO and SO treatment on IBD-related joint pain. Inhibition of COX is consistent with amelioration of IBD-related joint pain. WO appeared as effective as SO, but, as an active control group was used; further studies are required for proof of symptomatic effects.

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Captions to figures:

Figure 1. Plasma prostaglandin E2 level (ng/ml) before and after whale oil (WO) and seal oil (SO) administration. Boldface characters indicate significant differences.

Figure 2. Intensity of joint pain last week on visual analogue scale, VAS, in millimetre (mm) before and after whale oil (WO) and seal oil (SO) administration. Boldface characters indicate significant differences.

Figure 3. Intensity of back pain last week on visual analogue scale, VAS, in millimetre (mm) before and after whale oil (WO) and seal oil (SO) administration. Boldface characters indicate significant differences.

Figure 4. Total influence of pain last week on visual analogue scale, VAS, in millimetre (mm) before and after whale oil (WO) and seal oil (SO) administration. Boldface characters indicate significant differences.

Abstract

Compared with soy oil, 10 days' treatment with seal oil (SO), 10 mL × 3 daily, self-administrated through a nasoduodenal feeding tube, relieves joint pain in patients with inflammatory bowel disease (IBD). This randomized, controlled, double blind pilot trial compares SO and whale oil (WO) administered similarly by duodenal tube, for 10 days in 18 patients with IBD-related joint pain (n = 9 per group). Other long-chain n-3 polyunsaturated fatty acids were prohibited 7-days' prior to and during study. Significant changes from baseline to study end were observed in both groups: reduced plasma arachidonic acid to eicosapentaenoic acid ratio and prostaglandin E2 (PGE_2) levels (tendency in WO group), decreased IBD-related joint pain and IBD-disease activity, and improved quality of life. These changes were not significant between SO and WO groups. Inhibition of cyclooxygenase is consistent with amelioration of IBD-related joint pain, but, as active control was used, effects need confirmation.

Table

Table 1: Patient characteristics at baseline

Characteristic	WO group (n = 9)	SO group (n = 9)
Gender (male:female)	3:6	1:8
Mean age, yrs (range)	44.4 (31-58)	46.1 (31-57)
Mean disease duration intestinal disease, yrs (range)	14.4 (2-24)	12 (2-25)
Diagnoses		
Crohn's disease (CD)	4	4
Ulcerative colitis (UC)	5	5
IBD-associated arthritis	3	1
Arthralgia without diagnosed arthritis	6	8
Medical treatment		
NSAIDs (non-steroidal anti-inflammatory drugs)	3	0
DMARDs (disease-modifying anti-rheumatic drugs)	1[1]	0
Drugs that reduce bowel inflammation	6[2]	6[3]
Bowel surgery	5[4]	3[5]

WO = whale oil, SO = seal oil. Of the four patients diagnosed with IBD-associated arthritis, three had peripheral arthritis and one had undifferentiated spondyloarthropathy. Among the patients with arthralgia, one had been diagnosed with primary Sjögrens syndrome (WO group). [1]Sodium aurothiomalate, [2] Mesalazine 4, Sulfasalazine 1, Methotrexate 1, Prednisolone 1, [3]Mesalazine 4, Azathioprine 2, Sulfasalazine 1, [4] three patients with CD (multiple resections, one with stomi), two patients with UC (both ileoanal anastomosis with pelvic reservoir) and [5]three patients with CD (multiple resections).

Table

Table 2. Fatty acids, fat soluble vitamins and TBARS in experimental oils

	Whale oil	**Seal oil**
Fatty acid	**g/100 g**	**g/100 g**
Sum saturated	15.7	12.0
14:0	4.6	4.2
16:0	7.9	6.5
18:0	2.0	0.8
Sum monoenes	48.3	53.9
16:1 n-7	5.6	15.7
18:1 n-7	1.9	4.0
18:1 n-9	12.5	15.5
18:1 n-11	1.6	3.9
20:1 n-9	11.3	9.0
20:1 n-11	2.2	1.8
22:1 n-11	11.2	1.8
Sum n-6	2.5	2.3
18:2 n-6	1.7	1.7
20:4 n-6	0.4	0.5
Sum n-3	17.4	21.0
18:3 n-3	1.1	0.5
18:4 n-3	2.3	1.3
20:4 n-3	1.4	0.5
20:5 n-3	3.6	6.6
22:5 n-3	2.2	3.7
22:6 n-3	6.6	7.7
n-6/n-3	0.1	0.1
Vitamin A	n.d.	n.d.
Vitamin D	16 µg/100g	3 µg /100g
Vitamin E	2.2 mg/100g	58.3 mg/100g
TBARS	48.7 nmol/g w/w	39.6 nmol/g w/w

Vitamin A = sum retinol (13-, 11-, 9-cis and all trans retinol, i.e. A_1) and 3,4 didehydro-all-trans retinol (A_2). Vitamin D = vitamin D_3. Vitamin E = α-tocopherol. TBARS = thiobarbituric acid reactive substances. n.d. = not detected. w/w = wet weight.

Table

Table 3: Joint pain and IBD disease activity before and after treatment

	WO group		*P*-value	SO group		*P*-value
	Before	After		Before	After	
Morning stiffness (min)	25 (0-720)	7.5 (0-720)	0.13	30 (0-120)	22.5 (0-60)	0.13
Painful joints (number)	16.8 ± 2.9	9.6 ± 2.5	0.07	14.2 ± 3.4	12.9 ± 3.8	0.55
PainVAS (mm)	55.1 ± 3.9	33.6 ± 6.1	**0.02**	49.4 ± 6.9	27.9 ± 5.4	**0.002**
BackVAS (mm)	59 (2-87)	13 (1-59)	**0.03**	31 (0-65)	19 (0-40)	**0.02**
TotInfVAS (mm)*	64 (29-83)	43 (4-74)	**0.02**	43 (4-74)	26 (3-49)	**0.008**
IBD-index (score)	6.9 ± 1.4	5.6 ± 1.2	**0.02**	4.6 ± 0.9	3.2 ± 0.7	**0.049**
Calprotectin (mg/kg)	101 (20-1275)	128 (20-392)	0.38	47 (20-1275)	97.1 (20-287)	0.16

Mean ± SEM or median (range). WO = whale oil, SO = seal oil. Painful joints = numbers of painful joints. PainVAS = intensity of pain last week on visual analogue scale; BackVAS = intensity of back pain last week on visual analogue scale; TotInfVAS = total influence of pain last week on visual analogue scale; IBD-index = sum of Harvey-Bradshaw simple index for Crohn's disease and Walmsley simple clinical colitis activity index for ulcerative colitis; * = significant group difference at baseline ($P = 0.03$). *P*-values indicate change from before to after treatment in the WO or SO groups. Boldface numbers are statistically significant.

Table 4

Table 4: Plasma total fatty acid composition (μg fatty acid/g sample, w/w) before and after study

Fatty acid	WO group		*P*-value	SO group		*P*-value
	Before	After		Before	After	
14:0†	63 ± 7	67 ± 5	0.66	69 ± 12	42 ± 6	**0.01**
16:0†	1007 ± 75	948 ± 89	0.20	967 ± 69	715 ± 44	**0.0007**
18:0	265 ± 20	245 ± 20	0.07	255 ± 10	219 ± 7	**0.0008**
∑ saturated†	1357 ± 95	1283 ± 112	0.24	1315 ± 86	995 ± 51	**0.0007**
18:1 n-9	990 ± 99	871 ± 152	0.13	853 ± 69	588 ± 49	**0.0003**
∑ monoenes	1208 ± 113	1092 ± 168	0.19	1060 ± 91	763 ± 57	**0.001**
18:2 n-6†	1554 ± 132	1381 ± 100	**0.04**	1480 ± 70	1023 ± 68	**0.0007**
20:3 n-6	76 ± 6	48 ± 4	**<0.0001**	70 ± 8	37 ± 4	**0.0002**
20:4 n-6	282 ± 27	272 ± 22	0.32	297 ± 29	245 ± 24	0.06
∑ n-6†	1922 ± 155	1709 ± 117	**0.02**	1855 ± 77	1312 ± 88	**0.0009**
18:3 n-3†	35 ± 4	38 ± 2	0.37	39 ± 3	25 ± 3	**0.01**
20:5 n-3	47 ± 8	193 ± 26	**0.0002**	55 ± 11	244 ± 28	**0.0002**
22:5 n-3	25 ± 2	37 ± 3	**0.0002**	25 ± 3	34 ± 3	**0.007**
22:6 n-3	107 ± 16	215 ± 25	**0.0003**	111 ± 18	167 ± 12	**0.02**
∑ n-3	218 ± 27	507 ± 54	**<0.0001**	233 ± 30	477 ± 41	**0.0009**
n-6/n-3	9.2 ± 0.6	3.7 ± 0.4	**<0.0001**	8.6 ± 0.7	2.9 ± 0.2	**<0.0001**
20:4 n-6/20:5 n-3	6.6 ± 0.7	1.7 ± 0.3	**0.0002**	6.4 ± 0.7	1.1 ± 0.1	**<0.0001**
∑ Total fatty acids†	4831 ± 365	4692 ± 414	0.51	4705 ± 144	3577 ± 172	**0.0001**

Values are mean ± SEM. Sum total fatty acid include unidentified fatty acids. Percentage identified fatty acids ranged between 95.3-98.4. *P*-values indicate change from before to after treatment in the seal oil (SO) or whale oil (WO) groups. † = significant group difference after treatment. Boldface numbers are statistically significant. w/w = wet weight.

Figure 1

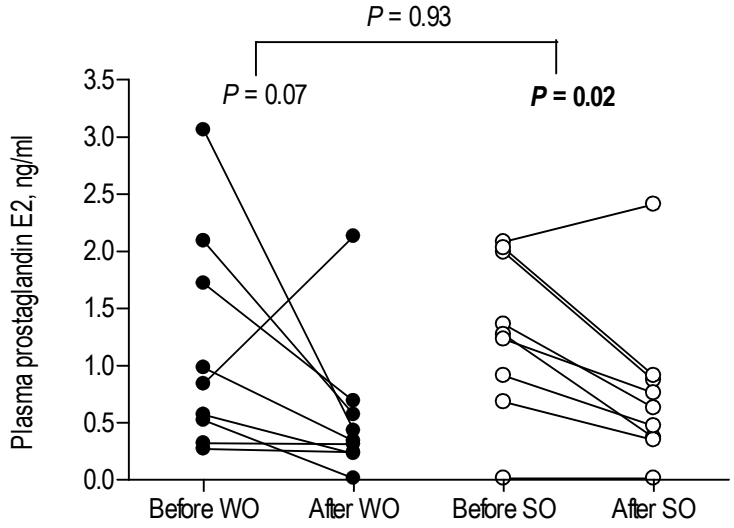

P = 0.93
P = 0.07
P = 0.02
Plasma prostaglandin E2, ng/ml
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Before WO
After WO
Before SO
After SO

Figure 2

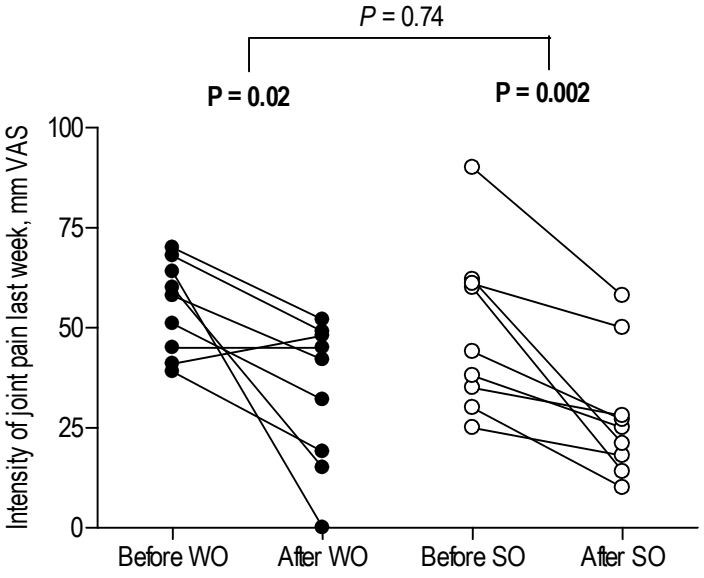

P = 0.74
P = 0.02
P = 0.002
Intensity of joint pain last week, mm VAS
100
75
50
25
0
Before WO
After WO
Before SO
After SO

Figure 3

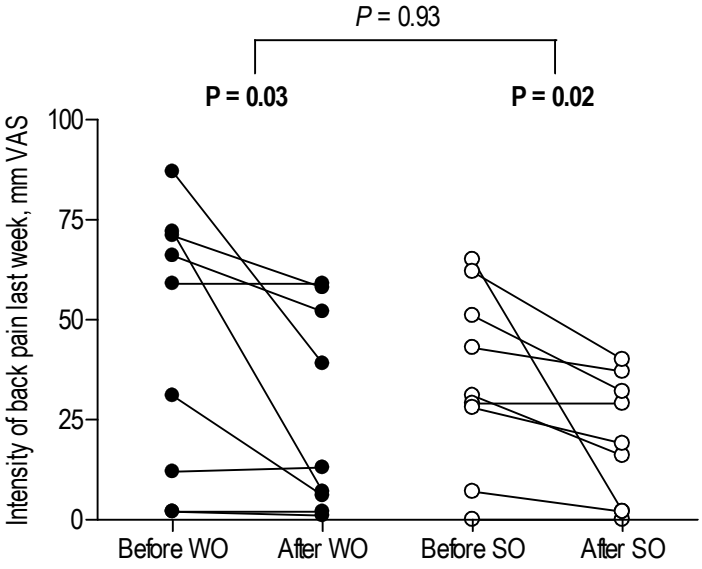
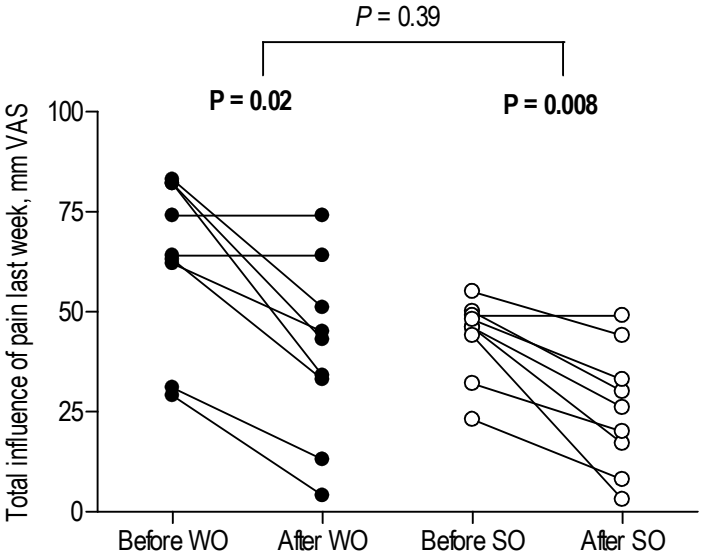

P = 0.93
P = 0.03
P = 0.02
Intensity of back pain last week, mm VAS
100
75
50
25
0
Before WO
After WO
Before SO
After SO

Figure 4


P = 0.39
P = 0.02
P = 0.008
Total influence of pain last week, mm VAS
100
75
50
25
0
Before WO
After WO
Before SO
After SO