Impact of marine oils in inflammatory bowel disease and psoriatic arthritis

With focus on effects of seal oil and whale oil on joint pain

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Scientific environment

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Tormod

Abstract

The Western diet has changed significantly over the last century, e.g. by an increased ratio of omega-6 (n-6) to n-3 polyunsaturated fatty acids (PUFA), particularly due to an increase in the use of vegetable oils like soy oil, rich in the n-6 PUFA linoleic acid (18:2n-6, LA), the precursor of arachidonic acid (20:4n-6, AA). Dietary fatty acids are incorporated into cell membranes of blood and tissues, where AA prevails as a result of the Western diet. AA and the long-chain (LC) n-3 PUFA eicosapentaenoic acid (20:5n-3, EPA), found in fatty fish and marine oils, compete as substrate for the synthesis of eicosanoids like e.g. prostaglandins by cyclooxygenase (COX) enzymes. AA-derived eicosanoids are generally more pro-inflammatory than their EPA-derived counterparts, and the former may be involved in both pathogenesis and exacerbation of chronic inflammatory diseases like inflammatory bowel disease (IBD) and rheumatic disorders.

Both IBD and psoriatic arthritis (PsA) patients suffer joint pain, but in contrast to PsA patients, IBD-patients commonly have arthralgia, i.e. joint pain without arthritis. Nevertheless, arthralgia substantially reduces the health related quality of life (HRQOL) of patients. Both IBD and PsA patients need safe alternative or adjuvant treatment for joint pain, as COX-inhibitory drugs for joint pain have adverse effects, particularly in IBD-patients. Long-term oral administration of fish oil reduces the levels of nociceptive prostaglandin E_2 (PGE_2) and is considered a moderately effective and safe strategy for ameliorating joint pain. In a recent pilot study in IBD-patients, 10 days administration of seal oil (SO), self-administrated as 10 mL × 3 daily by nasoduodenal feeding tube, reduced joint pain and IBD-disease activity. In the present thesis, eventual health benefits of administrating SO or whale oil (WO), 10 mL × 3 daily, with focus on joint pain relief, in patients with IBD and PsA, were investigated in explorative pilot studies.

Duodenal administration of SO for 10 days normalised n-6 to n-3 PUFA and AA to EPA ratios in rectal mucosa of IBD-patients as compared with controls. In a

later similar study in IBD-patients, SO ameliorated joint pain and HRQOL, the former with prolonged effects, as compared with soy oil, which tended to exacerbate the condition. In another similar study, both SO and WO reduced joint pain and IBD-disease activity and improved quality of life (QoL) in IBD-patients, with no significant group differences. The pain relief after SO and WO administration might in part be mediated by COX-inhibition as suggested by reduced PGE_2 levels in plasma (tendency only with WO) as analysed by liquid chromatography tandem mass spectrometry. The application of experimental design enabled estimating the interaction PGE_2/internal standard (IS) and selecting confidently an optimal amount of IS and a constant response factor in order to perform a reliable eicosanoid quantification.

In a rat model of IBD, short-term (seven days) pre-treatment with SO or cod liver oil (1 mL/day by gastric gavage) as supplement to a standard diet did not protect against subsequent dextran sulfate sodium (DSS) induced colitis, while soy oil aggravated the condition. In PsA patients, 14 days oral administration of SO reduced patients global assessment of disease four weeks post-treatment, but did not improve joint pain compared with soy oil. Twenty three % of PsA patients had elevated levels of faecal calprotectin suggesting asymptomatic, non-active colitis.

In conclusion, the present thesis suggests that short-term duodenal administration of blubber oils from marine mammals, like SO and WO, reduce joint pain and IBD-disease activity and improve QoL without significant adverse effects in IBD-patients with moderate disease activity, while short-term oral administration of SO does not improve joint or skin affections in PsA patients.

List of publications

I Bjørkkjær, T., Brunborg, L.A, Arslan, G., Lind, R.A., Brun, J.G., Valen, M., Klementsen, B., Berstad, A., & Frøyland, L. (2004): "Reduced joint pain after short-term duodenal administration of seal oil in patients with inflammatory bowel disease: Comparison with soy oil", Scand J Gastroenterol, 39:1088-94.

II Bjørkkjær, T., Brun, J.G., Valen, M., Arslan, G., Lind, R., Brunborg, L.A., Berstad, A., & Frøyland, L. (2006): **"**Short-term duodenal seal oil administration normalised n-6 to n-3 fatty acid ratio in rectal mucosa and ameliorated bodily pain in patients with inflammatory bowel disease", Lipids Health Dis, 5:6.

III Madland, T.M., Bjørkkjær, T., Brunborg L.A., Frøyland, L., Berstad, A., & and Brun, J.G. (2006): "Subjective improvement in patients with psoriatic arthritis after short-term oral treatment with seal oil. A pilot study with double blind comparison to soy oil", J Rheumatol, 33:307-10.

IV Arslan, G., Erichsen, K., Milde, A.M., Helgeland, L., Bjørkkjær, T., Frøyland, L., & Berstad, A. (2007): "No protection against DSS-induced colitis by short-term pretreatment with seal or fish oils in rats", Integrative Medicine Insights, 2:25-34.

V Araujo, P., Bjørkkjær, T., Berstad, A., & Frøyland, L. (2007): "Improved quantification of prostaglandins in biological samples by optimizing simultaneously the relationship eicosanoid/internal standard and using liquid chromatography tandem mass spectrometry", Prostaglandins Leukot Essent Fatty Acids, 77:9-13.

VI Bjørkkjær, T., Araujo, P., Madland, T.M., Berstad, A., & Frøyland, L. (in press 2009): "A randomized double blind comparison of short-term duodenally administrated whale and seal blubber oils in patients with inflammatory bowel disease and joint pain", Prostaglandins Leukot Essent Fatty Acids, doi:10.1016/j.plefa.2009.07.005.

Papers have been granted reprint permission from journals. In the thesis, these papers will be referred to by their Roman numerals.

Contents

Abbreviations

AA = arachidonic acid

ALA = α-linolenic acid

CD = Crohns' disease

COX = cyclooxygenase

CRP = C-reactive protein

DHA = docosahexaenoic acid

DPA = docosapentaenoic acid

DSS = dextran sulfate sodium

ELISA = enzyme-linked immunosorbent assay

EPA = eicosapentaenoic acid

FA = fatty acids

GI = gastrointestinal

GLC = gas liquid chromatography

GMP = granulocyte marker protein

HPLC = high performance liquid chromatography

HRQOL = health related quality of life

HUH = Haukeland University Hospital

IBD = inflammatory bowel disease

IS = internal standard

LA = linoleic acid

LCITMS = liquid chromatography ion trap mass spectrometry

LC-MS^n = liquid chromatography tandem mass spectrometry

LC n-3 PUFA = long chain omega-3 polyunsaturated fatty acid

LMF = Norwegian IBD-patient organisation

LOQ = limit of quantification

LOX = lipoxygenase

MHAQ = modified health assessment questionnaire

MUFA = monounsaturated fatty acids

NIFES = National Institute of Nutrition and Seafood Research

NSAIDs = non-steroidal anti-inflammatory drugs

PGE_2 = prostaglandin E_2

PASI = Psoriasis Area and Severity Index

PGE_2-*d4* = deuterated analogue of PGE_2 (internal standard, IS)

PsA = psoriatic arthritis

QoL = quality of life

RA = rheumatoid arthritis

RF = response factor

SF-36 = short-form 36

SF-NDI = short-form of the Nepean Dyspepsia Index

SO = seal oil

SpA = spondylarthritides

TAG = triacylglycerol

TBARS = thiobarbituric acid reactive substances

TNF-α = tumour necrosis factor-α

UC = ulcerative colitis

UoB = University of Bergen

VAS = visual analogue scale

WO = whale oil

w/w = wet weight

1. Background

The Western diet has changed significantly over the last century, e.g. by an increased ratio of omega-6 (n-6) to n-3 polyunsaturated fatty acids (PUFA) [1]. This is particularly due to an increase in the use of vegetable oils like soy oil, rich in the n-6 PUFA linoleic acid (18:2n-6, LA), the precursor of arachidonic acid (20:4n-6, AA). Dietary fatty acids (FA) are incorporated into cell membranes of blood and tissues, where AA prevails as a result of the Western diet [2]. AA and the long-chain (LC, i.e. 20 carbons or more) n-3 PUFA eicosapentaenoic acid (20:5n-3, EPA), found in fatty fish and marine oils, compete as substrate for the synthesis of eicosanoids like e.g. prostaglandins by cyclooxygenase (COX) enzymes. AA-derived eicosanoids are generally more pro-inflammatory than their EPA-derived counterparts, and the former may be involved in both pathogenesis and exacerbation of chronic inflammatory diseases like inflammatory bowel disease (IBD) and rheumatic disorders [3, 4]. Both IBD and psoriatic arthritis (PsA) patients suffer joint pain, but in contrast to PsA patients, IBD-patients commonly have arthralgia, i.e. joint pain without arthritis [5]. Nevertheless, arthralgia substantially reduces the health related quality of life (HRQOL) of patients [6]. Both IBD and PsA patients need safe alternative or adjuvant treatment for joint pain, as COX-inhibitory drugs for joint pain have adverse effects, particularly in IBD-patients [7]. Long-term oral administration of fish oil reduces the levels of nociceptive prostaglandin E_2 (PGE_2) and is considered a moderately effective and safe treatment for joint pain [4]. Less information exists on the potential health benefits of blubber oils from marine mammals, like seal oil (SO) and particularly whale oil (WO).

2. Aims of the study

2.1 Overall aim and context of thesis

The Royal Ministry of Fisheries and Coastal Affairs (RMFCA), the central governmental administrator of marine mammals in Norway, has emphasised the need for utilizing meat and blubber from sea mammals like seal and whale [8]. The overall aim of the present thesis was to document eventual health benefits of administrating SO or WO, with focus on joint pain relief, in patients with IBD and PsA. For a brief focus on potential mechanisms of effect of the oils administrated, the need for improved prostaglandin methodology was undertaken. Besides, for a brief focus on the impact of diet on the pathogenesis of IBD, an animal model was included. While tumour necrosis factor-α (TNF-α) inhibitors have recently been increasingly popular for treating inflammatory joint pain, they will not be discussed in this thesis. National Institute of Nutrition and Seafood Research (NIFES), the governmental research institute under RMFCA, with administrative duties devoted to seafood in human nutrition, has collaborated with Haukeland University Hospital (HUH) and University of Bergen (UoB) in this interdisciplinary project. Our strategy has been to provide generic information about SO and WO, not brand "X" or "Y", to the whole industry, governmental and non-governmental agencies, the research community and relevant user groups. Our approach has been documentation of nutrient content in, and health effects of, different commercial or non-commercial SO and WO openly, without any product development. Oils were either purchased or donated, the latter with no contractual limitations as to publication of eventual negative results. Only scientific aspects of SO and WO are elaborated on in the present thesis.

2.2 Aim of papers

Paper I: To compare the effects of short-term duodenal administration of SO versus soy oil on joint pain in patients with IBD and investigate potential prolonged effects during a six month follow-up period.

Paper II: To investigate whether normalisation of the n-6 to n-3 PUFA ratio in blood and tissues by SO is associated with improved HRQOL as assessed by the generic short-form 36 (SF-36) questionnaire.

Paper III: To compare the effects of short-term oral administration of SO versus soy oil on disease manifestations in PsA patients.

Paper IV: To investigate whether prior short-term supplementation with SO, cod liver oil or soy oil in addition to standard diet would influence the subsequent development of colonic inflammation in response to dextran sulfate sodium (DSS) in rats.

Paper V: To investigate how simultaneous changes of PGE_2 and internal standard (IS) concentrations affect the response factor (*RF*) and how modelling of the relationship PGE_2/IS can help select an optimal amount of IS in the analysis of plasma PGE_2 by liquid chromatography tandem mass spectrometry ($LC\text{-}MS^n$).

Paper VI: To compare the effects of short-term duodenal administration of WO versus SO on joint pain in patients with IBD.

3. Introduction

3.1 Inflammatory bowel disease

The two major forms of idiopathic IBD are Crohns' disease (CD) and ulcerative colitis (UC), both chronic enteric inflammatory diseases [9]. Flares with diarrhoea, abdominal pain and rectal bleeding in between periods with remission of symptoms are common. The aetiology is unknown; however, both genetics and environmental factors are involved in the pathogenesis. IBD is seemingly a result of an abnormal chronic activation of the mucosal immune system enhanced by luminal bacterial flora or alimentary allergens. Defects in the barrier function of the epithelium and the mucosal immune system are probably involved [10]. Although CD and UC are distinct diseases, they are not discrete, and share much of the same pathophysiology, treatment, complication and investigation.

CD may affect any part of the gastrointestinal (GI) tract; however only separated parts of the GI tract are inflamed (skip lesions of normal bowel wall in between inflamed areas). Distal ileum and proximal colon are affected in about two thirds of patients, while jejunum, duodenum, stomach and esophagus are infrequently affected. All layers of the GI wall may be affected, i.e. transmural inflammation. A prevalence of around 1.44 per 1000 has been reported for CD [11]. Indeed, the prevalence rates are increasing, particularly in Asia and among Asian immigrants living in Europe.

UC is characterized by continuous inflammation of the colonic mucosa only. Usually, the inflammation is most pronounced in the rectum and sometimes it is limited to the distal part of the colon only. A prevalence of 2.29 to 2.43 per 1000 has been reported for UC [11]. While not as much as for CD, the prevalence of UC is indeed increasing, particularly in Asia and among Asian immigrants living in Europe.

Figure 1 displays normal mucosa in healthy bowel and inflamed mucosa in CD and UC patients, respectively.

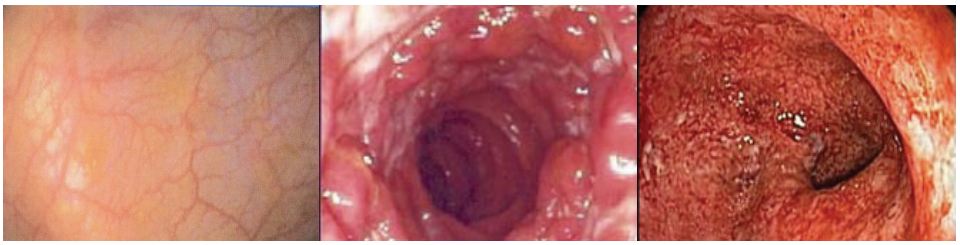

Figure 1. Normal mucosa in healthy bowel (left), inflamed mucosa in bowel of a Crohns' disease patient (middle) and inflamed mucosa in bowel of an ulcerative colitis patient (right). With permission from the Norwegian IBD-patient organisation (Landsforeningen mot fordøyelsessykdommer (LMF), Oslo, Norway).

3.1.1 Rheumatic complications of inflammatory bowel disease

Extraintestinal manifestations (EIM) can affect virtually every organ system including the joints, skin, mouth, liver and eyes and commonly occur in up to 40 % of IBD-patients and cause significant morbidity and distress for patients [12]. Rheumatic complications are the most common EIM, with peripheral arthritis present in 5-20 % of patients and axial arthritis (spondylitis or sacroiliitis) commonly present in 3-20 % of patients (sacroiliitis reported in up to 43 % of patients), as reviewed [12, 13]. Between 5-22 % of IBD-patients have joint pain without arthritis (i.e. arthralgia) [6, 14], though the prevalence may be higher as such patients are largely ignored due to few clinical or laboratory findings. Consequently, such patients are poorly treated despite experiencing disturbed sleep, painful joints, morning stiffness and reduced HRQOL [6].

3.1.2 Experimental models of inflammatory bowel disease

There are various animal (mainly rodent or murine) models of IBD, both spontaneous and induced models of acute and chronic inflammation, as reviewed [15]. Induction of experimental colitis by administration of toxic chemicals like e.g. acetic acid, indomethacin, trinitrobenzene sulfonic acid/ethanol or polysaccharides like DSS, carrageenan or immune complexes are most commonly used. Contrary to IBD in man, most of these models are not chronic and colonic healing is rapid [16]. When given in

drinking water (2-5 %) to rodents, DSS induce an acute colonic injury, with subsequent slow colonic regeneration and chronic colitis. DSS-induced colitis in the rat model results in inflammatory changes similar to those found in the colon of UC patients. The mechanism by which DSS induces colitis is not precisely known, but may involve a toxic effect directly on colonic epithelial cells.

3.2 Psoriatic arthritis

PsA is a heterogeneous chronic inflammatory joint disease, with five traditional subclasses as described in the initial Moll and Wright criteria [17]. Symmetric polyarthritis is increasingly the predominant subclass [18]. PsA is the second most prevalent chronic inflammatory joint disease in Caucasians, after rheumatoid arthritis (RA), with a prevalence of up to 1.95 per 1000 [18]. Skin and joint inflammation in psoriasis are related, but PsA is indeed distinct from psoriatic skin inflammation, as arthritis may be present without psoriasis in a few PsA patients [19]. Most PsA patients having arthritis also experience psoriasis, while only one fourth of psoriasis patients manifest arthritis, as reviewed [19]. Colonic mucosal inflammatory alterations [20] or even acute and chronic enteric inflammation [21, 22] are common in PsA patients. Interestingly, enteric inflammation may be involved in the pathogenesis of spondylarthritides (SpA) like PsA and IBD-associated joint complications [13, 23]. Figure 2 displays the hands of a patient with PsA.

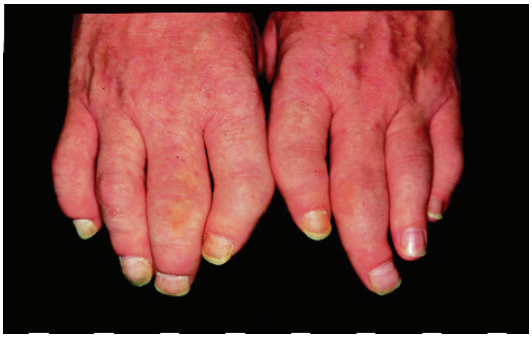

Figure 2. Hands of a patient with psoriatic arthritis. With permission from The Norwegian Psoriasis Federation (Norsk psoriasisforbund, Oslo, Norway).

3.3 Adverse effects of cyclooxygenase-inhibitors

Non-steroidal anti-inflammatory drugs (NSAIDs) are non-selective COX-inhibitors which target prostaglandins [24], as discussed later, widely prescribed and bought over-the-counter for rapid and effective treatment of joint pain. Unfortunately, the use of NSAIDs and possibly also the new selective COX-2 inhibitors are associated with GI adverse effects, e.g. barrier disruption, bleeding and ultimately ulcer disease, prompting a cautious use of such drugs [25]. This may particularly apply for IBD-patients, as COX-inhibitory drugs have been implicated in inducing IBD, activating quiescent IBD and exacerbating IBD-disease activity [7, 26]. Besides, the use of COX-2 inhibitors and NSAIDs increase the risk of serious adverse effects, e.g. fatal cardiovascular events [24, 27], recently resulting in the withdrawal of certain drugs from the market [28]. Thus, both IBD and PsA patients need safe alternative or adjuvant therapies for joint pain.

3.4 Nutritional aspects in relation to chronic inflammatory diseases

While the human genetic pool has remained much the same since the Paleolithic period 40 000 years ago (i.e. the birth of our ancestors), our diet has changed considerably [1], particularly during the last century. The current Western diet is characterised by an increase in total calorie intake (combined with low activity), saturated fat, trans-fatty acids, n-6 PUFA and cereal grains and a reduction in complex carbohydrates, fiber, protein, antioxidants, calcium, LC n-3 PUFA, fruits and vegetables. In the following, mainly n-6 and LC n-3 PUFA, present in vegetable and marine oils, respectively, are focused on.

3.4.1 Dietary fat and fatty acids

The fats in the human diet provide close to 40% of the energy intake in the Western world and the quantitatively most important lipid class is the triacylglycerol (TAG)

fraction, which is composed of a glycerol molecule with three FA attached [29]. As a result of the agricultural revolution, most of the dietary TAGs have increasingly been derived from cheap bulk-produced PUFA rich vegetable oils, particularly soy oil which is rich in LA, the precursor of AA [30]. Dietary FA are incorporated into blood and tissue cell membranes, which consist of a phospholipid bilayer. There are two principal PUFA families, namely the n-6 and the n-3 families, with LA and α-linolenic acid (18:3n-3, ALA) the simplest FA respectively [31]. LA and ALA are essential FA which need to be provided in the human diet and consequently are the precursors for the synthesis of longer chained and more unsaturated n-3 and n-6 PUFA through elongation and desaturation processes. The n-6 and n-3 PUFA families compete for the elongase and desaturase enzymes, which have greater affinity for substrates with many double bonds like n-3 PUFA. However, as a result of a high dietary n-6 to n-3 PUFA ratio in the Western diet, AA prevails in cell membranes of blood and tissues [2] and n-3 PUFAs are naturally less available for the above mentioned enzymes [30]. Besides, the *in vivo* conversion of ALA to EPA, docosapentaenoic acid (22:5n-3) and particularly to docosahexaenoic acid (22:6n-3, DHA) are limited in humans [32]. Thus, ingestion of preformed LC n-3 PUFAs like EPA, DPA and DHA, commonly referred to as “marine” due to their abundance in fatty fish, seafood and marine oils, is considered highly important, as reviewed [33].

3.4.2 N-6 and long chain n-3 polyunsaturated fatty acids as precursors of eicosanoids

A high dietary ratio of n-6 to n-3 PUFA may result in an imbalance in the ratio of AA to EPA, which is rate limiting for the production of eicosanoids, hormone-like metabolic compounds [1]. AA and EPA liberated from cell membranes by phospholipases compete as substrate for the synthesis of e.g. prostaglandins and thromboxanes by COX and leukotrienes by lipoxygenase (LOX) enzymes, and are thus precursors of these eicosanoids. Prostaglandins derived from AA generally induce arrhythmia, while the opposite is the case for their EPA derived counterparts. Besides, prostaglandins like PGE_2 are implicated in pain [24]. In general, leukotrienes derived

from AA are more pro-inflammatory than their EPA-derived counterparts, which may in fact be anti-inflammatory. In general, thromboxanes derived from AA activate platelets and are vasoconstrictors, while thromboxanes derived from EPA are platelet inhibitors and vasodilators. Administration of marine oils partially replace AA with EPA and DHA in cell membranes, and thereby reduce levels of generally pro-inflammatory eicosanoids like PGE_2 and leukotriene B_4 (LTB_4) derived from AA [3]. Besides, EPA is substrate for less pro-inflammatory eicosanoids like e.g. prostaglandin E_3 (PGE_3) and leukotriene B_5 (LTB_5). Antagonising AA metabolism through altered eicosanoid production is a key anti-inflammatory effect of LC n-3 PUFA, while other anti-inflammatory effects dependent or independent of eicosanoid production have also been described [3].

3.4.3 Western diet in relation to pathogenesis of chronic inflammatory diseases

In an evolutionary aspects, it is notable that the diet of our ancestors had an n-6 to n-3 PUFA ratio of close to 1:1, while today the ratio is commonly 15-16.7:1 in the Western world [34]. The general dietary recommendation over the last 50 years has been “to eat some PUFA”, based on limited documentation on potential health implications [35]. Indeed, a high dietary n-6 to n-3 PUFA ratio has been linked to the increased prevalence of chronic inflammatory diseases like e.g. IBD and RA [36], while little is known about diet in relation to the pathogenesis of PsA. Interestingly, a high n-6 PUFA intake in early life stages, and even during fetal exposure, may trigger an abnormal colonic inflammatory response when exposed to a GI insult later in life [37]. In order to possibly slow down and prevent this development of numerous chronic inflammatory diseases, lowering the n-6 to n-3 PUFA ratio in our diet is essential [30]. Indeed, recently more specific PUFA recommendations like increasing the LC n-3 PUFA intake have been enforced [35].

3.4.4 Marine oils

The traditional Eskimo diet contained traditional food, little processed food and seafood in general, especially meat and blubber from marine mammals like e.g. seals and whales [38]. However, most research has focused on fish oils and particularly its high content of EPA and DHA ever since the initial studies by Dyerberg and Bang [39]. Interestingly, a low prevalence of common westernized diseases like e.g. IBD, bronchial asthma, multiple sclerosis, RA and psoriasis were previously seen in Greenland Eskimos [40, 41], while today the Western diet is much more common and the prevalence of many typical westernized diseases are increasing [42].

Compared to fish oil, SO and particularly WO contains less EPA and DHA but more of DPA, a potent inhibitor of platelet aggregation [43], as confirmed when administrating these oils to humans or animals [27, 44, 45]. SO and WO are structurally different oils compared to fish oil. EPA and DHA are almost exclusively located in the *sn*-1 or *sn*-3 (outer) positions of TAG from SO and WO, while these FA are located mainly in *sn*-2 (middle) position of TAG from fish oil [46]. While FA in the *sn-2* position of TAG are generally preserved (75 %) through digestion and absorption [47], and intramolecular distribution of FA in TAG generally do not influence tissue uptake [29, 48], *sn-1*/*sn-3* position specific lipoprotein lipases [49] 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 [50]. Whether this also applies for natural TAGs with complex FA compositions remains to be elucidated.

Marine oils on the market should as any other food for human consumption comply with regulations on contaminants, implying removing the contaminants by conventional refining or molecular distillation in the case of marine oils. Such processing may negatively influence the level of nutrients, natural antioxidants and other bioactive components and consequently the quality of such products [51]. Levels of fat soluble vitamins A, D and E are normally reduced during this cleaning process; however antioxidants or tocopherols are commonly added for protection of oils against lipid peroxidation. Vitamin D is added in some oils, most notably in cod liver oil.

3.4.5 Effects of marine oils in some chronic inflammatory diseases

Long-term oral administration of LC n-3 PUFA has been shown to reduce enteric and skin inflammation in clinical studies of IBD [52] and psoriasis [53], respectively, however effects are inconsistent. In comparison, fish oil administration generally reduces colonic inflammation and damage, weight loss and mortality in animal models of colitis [3].

Effects on IBD- and PsA-related joint pain have been scarcely studied. In a recent open pilot study, short-term (10 days) nasoduodenal administration of SO, 10 mL × 3 daily, reduced joint pain and IBD-disease activity without adverse effects in patients with mild to moderately active IBD [54], while the effect of WO is unknown. In a previous study of 80 patients with stable chronic psoriasis, 43 % (34 patients) with PsA, joint pain and PASI (Psoriasis Area and Severity Index) scores were reduced after 8 weeks administration of capsules with 1.1 g EPA and 0.8 g DHA ethyl esters daily [55]. In a later study, patients with chronic stable plaque psoriasis and PsA were given Efamol Marine capsules with 480 mg γ-linoleic acid, 240 mg EPA and 132 mg DHA daily or placebo for 9 months, followed by placebo for three months to both groups [56]. No clinical joint or skin effects were seen, possibly due to the low daily FA dosage.

In RA patients, long-term (at least 3 months) oral administration of fish oil moderately reduces joint pain, morning stiffness, number of painful and/or tender joints and NSAID consumption [57]. Besides, marine oil administration has beneficial cardiovascular “side-effects” [27, 44], suggesting treatment of joint pain may have collateral health benefit in RA and possibly PsA patients, which have increased risk of cardiovascular disease [58]. Importantly, fish oil is a natural COX-inhibitor which clearly outweighs NSAIDs regarding sum of benefit and risk [59]. Still, NSAIDs are widely used, while fish oils are scarcely used, possibly due to the latency of effect of orally administrated marine oils but also due to marketing issues [4].

3.5 Methodology for the analysis of eicosanoids

Traditionally, the analysis of eicosanoids is performed by using immunological assays, e.g. enzyme- or radio-immunoassays. Although immunological assays are inexpensive and simple to perform, their main disadvantages are the associated high variability, the lack of specificity for complex biological matrices, the potential cross-reactivity and consequently the analytes overestimation [60, 61].

Single or coupled chromatographic methods are increasingly being used for the analysis of eicosanoids [62]. LC-MS^n may provide a powerful tool for selectively and accurately analysing prostaglandins. However, its major drawback is a limit of quantification (LOQ, in ng/ml) higher than those reported by sensitive immunological assays (LOQ in pg/ml) and LC-MS^n is consequently unable to estimate normal occurring endogenous levels of PGE_2 in plasma [63]. Although this disadvantage of LC-MS^n, it is a suitable technique in clinical studies where pathological levels of PGE_2 are assessed.

LC-MS^n quantitative studies involving the external standard calibration (a technique currently used in immunological assays) and/or a large number of samples to be analysed, are impractical, difficult and time consuming and call for compulsory construction of several calibration curves. To overcome these problems, the IS technique is the preferred approach to attain rapid sample analysis efficiency at a minimum cost. The IS approach consists of adding deliberately a compound resembling the eicosanoid of interest to the sample. When available, the ideal IS is a deuterated analogue of the analytical prostaglandin.

4. Material and methods

4.1 Inflammatory bowel disease studies (papers I, II and VI)

Consecutive out-patients with IBD, i.e. CD or UC, and joint pain with or without arthritis (the latter arthralgia), were recruited at Department of Medicine, Section for Gastroenterology and Department of Rheumatology, both HUH, and through the Norwegian IBD-patient organisation (LMF, Oslo, Norway). Patients generally had a history of joint pain and as clinical effects appeared similar in CD and UC patients, these patient groups were pooled and referred to as patients with IBD-related joint pain. In papers I, II and VI the patients were randomly allocated to 10 days treatment with marine or vegetable oils (10 mL × 3 daily before meals), self-administrated through a nasoduodenal feeding tube (Freka® Feeding Tube, Fresenius Kabi, GmbH, Germany), with a six month follow-up period in paper I (Table 1). The feeding tube in question is 120 cm long and made of polyurethane (2.1 mm internal diameter and 2.8 mm outer diameter), originally designed for up to 4-6 week enteral nutrition purpose. Figure 3 shows a nasojejunal feeding tube, located with its tip in the jejunum, not the proximal part of duodenum as in our case. In paper II, control samples were obtained during routine biopsies of prostate cancer patients without IBD or joint pain (Table 1).

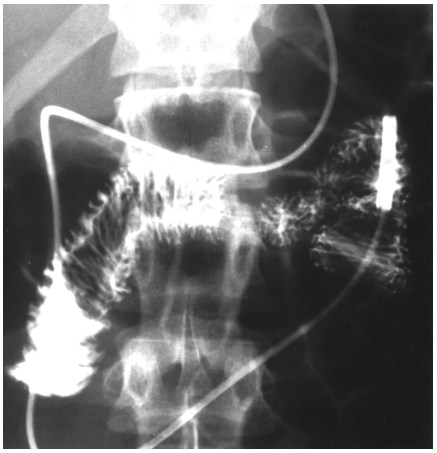

Figure 3. Fluoroscopy image of an inserted nasojejunal feeding tube. With permission from Professor Arnold Berstad, University of Bergen/Haukeland University Hospital.

4.2 Prostaglandin E_2 method study (paper V)

Plasma samples from 21 IBD-patients in paper VI were used in a PGE_2 method study. Considering the expected variable levels of PGE_2 in the clinical samples, it was decided to optimise firstly the amount of IS (PGE_2d_4) using the experimental design approach illustrated in Figure 1 of paper V, where blank plasma samples were spiked with known amounts of PGE_2 and PGE_2-d_4 and quantified by means of LC-MS^n as described later. To this aim, the concentrations of PGE_2 and IS were varied simultaneously as detailed in paper V and the behaviour of the response factor (*RF*) was studied and determined by the expression: $RF = \frac{ng_{PGE_2}}{ng_{PGE_2d_4}} \times \frac{S_{PGE_2d_4}}{S_{PGE_2}}$ (Eq. 1)

The term *ng* in Eq. 1 above refers to nanograms of PGE_2 and PGE_2d_4 injected in the liquid chromatography system and the term *S* to the analytical signals monitored in the mass spectrometry detector and recorded in ion counts per seconds.

4.3 Psoriatic arthritis study (paper III)

Among all PsA patients (n = 634) identified in the county Hordaland, Norway in the period 1999-2002 [18], those with polyarticular PsA and seen with active joint disease at Department of Rheumatology, HUH during the last year were sent a written request to attend 14 days oral administration of marine or vegetable oils (10 mL × 3 daily before meals) with a four week follow-up period (Table 1).

4.4 Experimental colitis study (paper IV)

Forty-eight adult male Wistar rats were divided into 6 groups: no intervention, sham (distilled water), DSS, seal oil + DSS, cod liver oil + DSS and soy oil + DSS (Table 1). Following one week of acclimatisation, 1 mL oil/day (seal, cod liver or soy) or sham was administered by gastric gavage to rats for one week, followed by induction of colitis by 5% DSS in drinking water for one week.

Table 1. Overview of material, study design and methods in papers I-IV and VI

	Paper I	Paper II	Paper III	Paper IV	Paper VI
Patients/animals	IBD-related joint pain (n=19)	1) IBD (n=10, 9 with joint pain) and prostate cancer (n=10) 2) IBD-related joint pain (n=17)	PsA (n=40)	Adult male Wistar rats (n=48)	IBD-related joint pain (n=18)
Experimental group(s)	SO (n=10)	1) SO to IBD-patients 2) SO	SO (n=20)	SO + DSS (n=8), CLO + DSS (n=8), VO + DSS (n=8), DSS (n=8)	WO (n=9)
Control/placebo group	VO (n=9)	1) No oil to controls 2) VO	VO (n=20)	No oil (n=8), sham (n=8)	SO (n=9)
Mode of oil administration (daily dosage)	Duodenal (10 mL × 3)	Duodenal (10 mL × 3)	Oral (10 mL × 3)	Gastric gavage (1 mL)	Duodenal (10 mL × 3)
Duration of oil administration + follow up	10 days + 1, 2, 4 and 6 month	1) 10 days (IBD-patients) 2) 10 days + 1, 2, 4 and 6 month	14 days + 1 month	Oils/no oils/sham for 7 days before DSS-induced colitis	10 days
Joint measures	Morning stiffness; tender, swollen and painful joints; VAS (3 different); MHAQ		Tender and swollen joints; VAS (2 different); MHAQ		Morning stiffness, painful joints, VAS (3 different)
Intestinal measures	Faecal calprotectin + IBD-index		Faecal calprotectin at baseline	Faecal GMP (day 22) + colonic histology (day 23)	Faecal calprotectin + IBD index
Skin measures			PASI		
Quality of life questionnaire		2) SF-36			SF-NDI
Diet assessment (baseline)	Questionnaire on fish and LC n-3 PUFA supplements	1) Not performed 2) Questionnaire on fish and LC n-3 PUFA supplements	Habitual intake of fish and fish oil reported	Data on standard diet	Habitual intake of fish and LC n-3 PUFA supplements reported (diet restrictions)
Fatty acid analysis	Serum before and after treatment	1) Rectal mucosal biopsies before and after SO (IBD-patients) or one sample from controls	Serum	RBC at day 22	Plasma
Other measures	Serum TBARS before and after treatment		-Serum: cholesterol, homocysteine, ESR, CRP and TBARS -Plasma: calprotectin -Blinding rate (after)	-DSS intake (every other day) -BW (day 8, 15, 23) -Colon length (day 23)	-Plasma PGE_2 -Preferences (before) -Blinding rate (after) -AE questionnaire

Paper II: 1) = data from the pilot study on seal oil in IBD [54] and 2) = data from paper I. Assessments/analysis before and after study and during follow-up unless specified. TBARS = thiobarbituric acid reactive substances. ESR = erythrocyte sedimentation rate. CRP = C-reactive protein. IBD = inflammatory bowel disease. PsA = psoriatic arthritis. VAS = visual analogue scale. MHAQ = modified health assessment questionnaire. DSS = dextran sulfate sodium. GMP = granulocyte marker protein. IBD-index = questionnaire on IBD-disease activity. PASI = psoriasis area and severity index. AE = adverse effects. PGE_2 = prostaglandin E_2. SO = seal oil. CLO = cod liver oil. VO = vegetable oil (soy oil). WO = whale oil. BW = body weight. SF-NDI = short-form Nepean dyspepsia index. LC n-3 PUFA = long chain omega-3 polyunsaturated fatty acids.

4.5 Marine and vegetable oils

One crude and three refined (conventional process) products of SO were used in the clinical and animal studies (papers I-IV and VI). The refined SO was from adult harp seals, and the crude SO was a mixture of oil from adult harp and hooded seals, all of Canadian origin. The fish oil used in paper IV was a commercially available refined (conventional process) cod liver oil and the WO (from adult minke whales) used in paper VI was a molecularly distilled (slow speed at 195°C) oil, both Norwegian products. As marine mammals are atop the marine food chain, levels of contaminants were analysed in all marine oils to satisfy current European Union (EU) regulations on contaminants. The contribution of contaminants in oils to the tolerable weekly intake (TWI) was estimated before use in clinical studies.

While no antioxidant was added to the crude SO (i.e. containing only natural tocopherols), a combination of natural and synthetic tocopherols, the latter dl-α tocopheryl acetate, was added to SO in papers II and III and in cod liver oil in paper IV. In paper VI, natural tocopherols were added to SO and dl-α tocopheryl acetate was added to WO.

Refined commercially available soy oil from one manufacturer was used in papers I-IV. See Table 2 for an overview of fatty acids, fat soluble vitamins (A, D and E) and lipid peroxidation (thiobarbituric acid reactive substances, TBARS) in the marine and vegetable oils used in papers I-IV and VI. Marine oils were stored frozen at -20°C until used in clinical trials, while soy oil was bought fresh. Oils used in clinical trials were distributed in regular polypropylene bottles with nitrogen on top, and stored in refrigerator (0-4°C) during study.

Table 2. Fatty acid profile (g/100 g), vitamins A, D and E and TBARS in experimental oils

Analyte	Crude SO[I, II, IV]	Refined SO[II]	Refined SO[III]	Refined SO[VI]	Cod liver oil[IV]	Whale oil[VI]	Soy oil[I-IV]
14:0	4.2	4.4	4.5	4.2	4.0	4.6	n.d
16:0	7.5	7.1	8.0	6.5	8.9	7.9	9.7
18:0	1.0	0.8	1.2	0.8	1.8	2.0	3.0
∑ saturated	12.9	12.7	14.2	12.0	15.4	15.7	13.4
16:1n-7	11.7	9.7	14	15.7	6.1	5.6	n.d
18:1n-11	1.9	1.5	3.2	3.9	2.0	3.9	n.d
18:1n-9	16.3	14.7	14.9	15.5	13.7	12.5	17.5
18:1n-7	3.9	3.4	3.8	4.0	3.3	4.0	1.3
20:1n-11	1.3	1.2	1.6	1.8	1.6	2.2	n.d
20:1n-9	7.5	8.1	7.7	9.0	10.2	11.3	0.2
22:1n-11	2.6	3.7	1.8	1.8	7.5	11.2	n.d
∑ monoenes	47.6	44.2	48.9	53.9	46.0	48.3	19.0
18:2n-6	1.4	1.5	1.5	1.7	1.5	1.7	49.7
20:4n-6	0.4	0.4	0.6	0.5	0.4	0.4	n.d
∑ n-6	1.8	2.1	2.2	2.3	2.2	2.1	49.7
18:3n-3	0.7	0.8	0.6	0.5	0.8	1.1	5.5
18:4n-3	2.1	2.6	1.6	1.3	2.3	2.3	n.d
20:4n-3	0.5	0.5	0.5	0.5	0.7	1.4	n.d
20:5n-3	6.6	5.8	7.9	6.6	7.5	3.6	n.d
22:5n-3	3.1	3.3	3.7	3.7	1.1	2.2	n.d
22:6n-3	7.4	8.9	8.6	7.7	12.4	6.6	n.d
∑ n-3	21.0	22.1	23.9	21.0	25.1	17.8	5.5
n-6/n-3	0.1	0.1	0.1	0.1	0.1	0.1	9.0
Sum vitamin A	1.4 mg/100g	1.1 mg/100g	0.3 mg/100g	n.d	4.8 mg/100g	n.d	n.d
Vitamin D_3	17 µg/100g	n.a.	n.a.	3 µg/100g	242.7 µg/100g	16 µg/100g	15 µg/100g
α-tocopherol	5.4 mg/100g	5.4 mg/100g	4.5 mg/100g	58.3 mg/100g	309 mg/100g	2.2 mg/100g	17.1 mg/100g
TBARS	15.3 nmol/g w/w	2 nmol/g w/w	3.6 nmol/g w/w	39.6 nmol/g w/w	13.8 nmol/g w/w	48.7 nmol/g w/w	n.d

Monoenes = monounsaturated fatty acids. Sum 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). TBARS = thiobarbituric acid reactive substances. n.d = not detected. n.a = not analysed. w/w = wet weight. SO = seal oil. Roman numerals in relation to oils refer to which paper (s) they have been used in. Values are mean of two analytical replicates.

4.6 Assessments and questionnaires

As estimates of disease activity, patients reported the mean duration of morning stiffness last week (maximum 720 min), i.e. generally how long until musculoskeletal stiffness "loosens up". Using a horizontal visual analogue scale (VAS) ranging from zero (very well) to 100 (very poor), various pain or disease activity measures (mean of last week) were assessed by patients or a rheumatologist [64].

In the clinical IBD-studies, a reduced 28-joint count [65] consisting of 10 proximal interphalangeal (PIP) joints, 10 metacarpophalangeal (MCP) joints, wrists, elbows, shoulders and knees was used for painful (patient assessed), (palpation) tender and swollen joints, as assessed by a rheumatologist. In addition to this simplified joint count, validated in RA patients [65], ankles and toes (scored as one, i.e. totally 40 joints) were assessed. In the PsA study, we used the EULAR (European League Against Rheumatism) 44 joint count (i.e. the above mentioned 40 joints plus two collar bone joints on both sides) [66] together with distal interphalangeal (DIP) joints of fingers; i.e. 52 joints in total. Functional level of joint disease was assessed with a modified health assessment questionnaire (MHAQ) in papers I and III, with values ranging from 1 (no problem to perform) to 4 (impossible to perform) [67].

Harvey-Bradshaw simple index [68] and Walmsley simple clinical colitis activity index [69], largely subjective but valuable measures of IBD disease activity [70], were used in CD and UC patients respectively. A score equal to or higher than six indicates active IBD. Scores for CD and UC disease activity index were pooled in results as previously [54, 71]. The PASI score was used for assessing skin manifestations in PsA patients, ranging from 0 (no psoriatic lesions at all) to 72 (complete erythroderma of the severest possible degree), with 0.1 unit steps [72], and levels above 10 normally requiring hospitalisation.

The Medical Outcome Study (MOS) SF-36 health survey questionnaire was used for assessment of HRQOL in paper II [73]. A Norwegian translated version was used, validated in RA patients [74, 75]. Final values ranged from 0 (very poor) to 100 (very well). In paper VI, quality of life (QoL) was assessed with the short-form of the Nepean Dyspepsia Index (SF-NDI), originally developed for functional dyspepsia

patients [76]. A Norwegian translated version was used, validated in patients with food hypersensitivity [77].

Background diet was briefly assessed throughout the clinical studies. In paper I, a simple self-administered questionnaire on marine food (LC n-3 PUFA supplements and fish) was used. Additional results from paper I and from the pilot study [54], the latter with no diet evaluation, were presented in paper II. In paper III, patients recorded intake of fish and seafood as "never", "approximately once weekly" or "twice or more per week" in addition to LC n-3 PUFA supplements. In paper VI, patients' habitual seafood intake was reported descriptively in brief. In all clinical studies, patients were asked to continue their normal Western diet throughout study. In the animal study, nutrients in standard diet (pellets) as specified from manufacturer were given. Besides, rats were weighed and intake of DSS-water measured.

In paper VI, patients were asked about preference before study (i.e. if they preferred one particular oil or not) and blinding rate (how many patients who correctly identified which oil they received) was assessed in papers III and VI. In paper III, those who did not know and those who guessed incorrect were pooled. Self reported adverse effects were noted in clinical trials, while additionally a questionnaire on adverse effects was answered by patients in paper VI.

4.7 Laboratory analysis

Venous blood samples from fasting patients (non-fasting patients in paper III) or following exsanguination of fasted rats were collected in vials with no anticoagulant (gel vials for serum) or with anticoagulant (for plasma), centrifuged and stored at -80°C prior to analysis of FA 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, in a final concentration of 10 µg indomethacin/mL plasma, within 30 minutes of venupuncture. Replicate aliquots of plasma without indomethacin were taken for control.

FA composition of total lipids was analysed by gas liquid chromatography (GLC) at NIFES in blood and tissues (for compliance purpose) and marine oils as described previously [78], with some modifications as given in papers I-IV and VI. In brief, total lipid content was extracted, filtered and evaporated, sample saponified and FA were esterified. The methyl esters were separated using a gas chromatograph as described in papers I-IV and VI, equipped with a 50 m CP sil 88 (Chrompack) fused silica capillary column (id: 0.32 mm), using "cold on column" injection, with a temperature programme of $60^{25°C/min}160^{25°C/min}190^{25°C/min}220°C^{5min}$ and flame ionization detector. The FA 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 IS for quantitative determination of FA. LOQ was 10 µg FA/g sample (wet weight, w/w).

In paper VI, PGE_2 was extracted from plasma with and without added indomethacin and analysed by using a LC-MS^n method developed at NIFES for the analysis of this analyte in human plasma [79] with modifications given in paper V. 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 volume 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*, the deuterated analogue of PGE_2, was used as IS. LOQ was 0.4 ng PGE_2/ml.

In paper III, erythrocyte sedimentation rate (ESR) was analysed according to Westergren method, C-reactive protein (CRP), total plasma homocysteine, total-, high-density lipoprotein (HDL) - and low-density lipoprotein (LDL)-cholesterol (all three Roche Modular enzymatic kits) were analysed by routine methods at Laboratory of

Clinical Biochemistry, HUH. Plasma calprotectin was analysed by enzyme-linked immunosorbent assay (ELISA) at Ullevaal University Hospital, with a reference interval of 100-900 µg/L [80]. Calprotectin is a calcium- and zinc-binding protein, and blood levels of calprotectin reflects disease activity in PsA and other arthritides [81].

Faecal calprotectin, a non-invasive biomarker of IBD disease activity, was analysed in frozen spot samples of faeces in clinical IBD-studies and PsA study by ELISA, at the Section for Gastroenterology, Institute of Medicine, UoB. Calprotectin is the main soluble protein present in neutrophil granulocytes, monocytes and macrophages in the intestine [82]. Generally, a faecal calprotectin value above 50 mg/kg is abnormal, but in clinical practice, only values above 500 mg/kg indicate active IBD [82].

Faecal granulocyte marker protein (GMP), a rat counterpart of human calprotectin, was analysed by ELISA, as a marker of disease activity in experimental colitis, with levels up to 25 mg/L considered normal [83]. Histological assessment of colonic crypt and inflammatory scores in formalin fixed, paraffin-embedded colonic segments, stained with haematoxylin and eosin, were done according to a validated scoring system [84] after recording the length of the colon.

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), were analysed in marine oils at NIFES by a modified high performance liquid chromatography (HPLC) method [85, 86]. 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 ultra violet (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 at NIFES by HPLC as described previously [87]. In brief, sample material is saponified and the unsaponified material is extracted before clean-up on a preparative column (HICHROM, Kromasil silica, 5 µm, 4.6 × 250 mm). The fractions with vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol) are 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 was determined by UV-detector (LaChrom, Merck HITACHI L-7420) and quantified using vitamin D_2 as IS. LOQ was 1 µg vitamin D_3/100 g sample (w/w).

Vitamin E in marine oils was analysed at NIFES by HPLC, based on the principles reported by European Committee for Standardization (CEN) [88]. 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 analysed in serum and marine oils at NIFES by a modified in vitro method, measuring mainly malondialdehyde and other aldehydes, secondary lipid peroxidation parameters [89, 90]. In brief, fat and water-soluble components are separated, while the analyte is extracted in a 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. LOQ was 3.9 nmol TBARS/g sample (w/w). Serum TBARS values in PsA patients were generally below LOQ and therefore not reported in paper III.

4.8 Statistics

Data were presented and normality tested as detailed in papers. Generally, group differences were compared by unpaired Student's t-test or Mann Whitney, while paired differences were evaluated by paired t-test or Wilcoxon sign rank test. In papers I and II, area under the curve (AUC) for the entire period from start of treatment until 6 months post-treatment was calculated with the trapezoid method. In paper III,

monovariate associations between variables were analysed using the Spearman rank order correlation test. In paper IV, differences between means were evaluated with one-way ANOVA (analysis of variance) and post-test (Bonferroni) for selected pairs of columns. In paper V, a multiple regression analysis was performed and the statistical significance of the coefficients and the correlation was determined by the *F*-test. Descriptive statistics was used when appropriate. Except for a 99 % confidence level in paper III, *P* values < 0.05 were generally regarded as statistically significant. Statistical data-analysis and displaying of figures were performed using the GraphPad Prism 4 (GraphPad Software Inc, San Diego, USA) statistical software package in papers I, II, IV and VI. In paper III, SPSS release 13.0 software (SPSS Inc., Chicago, USA) was used and in paper V, Statgraphics Plus 5.1 software package (Statpoint Inc., Virginia, USA) was used for statistical data-analysis. Microsoft®Excel 2002 (Microsoft Corporation, Washington, USA) was used for displaying of figures in paper V.

4.9 Ethics

The clinical trials (papers I-III and VI) were performed according to the Declaration of Helsinki, after approval from the Regional Committee for Medical Research Ethics and written informed consent from patients (also from the parents of one adolescent included in paper I). An application was sent to the Norwegian Social Science Data Services regarding data protection, and biological samples were stored in an approved research biobank at NIFES. The blood samples obtained from patients in paper VI were approved for research, and used during method development in paper V. The animal study (paper IV) was performed by Federation of European Laboratory Animal Science Associations (FELASA) trained personnel in accordance with The Norwegian Animal Welfare Act after approval from the Norwegian Animal Research Authority.

5. Results

5.1 Paper I: Serum ratios of n-6 to n-3 PUFA and AA to EPA were reduced after 10 days duodenal administration of SO. Compared with similar administration of soy oil, SO significantly reduced the duration of morning stiffness, number of tender joints, intensity of pain and the doctor's scoring of rheumatic disease activity. AUC for the entire period from start of treatment until 6 months post treatment, suggested a long-lasting beneficial effect of morning stiffness, MHAQ and doctor's scoring of rheumatic disease activity after SO administration, while soy oil administration tended to aggravate the condition (not significantly). No patients had faecal calprotectin levels above 50 mg/kg at inclusion, while one patient in the SO group had a value of 76 mg/kg after treatment.

5.2 Paper II: In the pilot study [54], 10 days duodenal administration of SO normalised ratios of n-6 to n-3 PUFA and AA to EPA in rectal mucosa of IBD-patients compared with levels found in controls. Similar administration of SO reduced the bodily pain dimension of HRQOL compared with soy oil in paper I.

5.3 Paper III: There was a fall in the ratios of n-6 to n-3 PUFA and AA to EPA in serum after 14 days oral treatment with SO, but no effect on joint pain or skin affections compared with soy oil. Patients in the SO group reported a significant improvement in global assessment of the disease four weeks post-treatment, with no significant group differences. At four weeks post-treatment, non-significant tendencies of decreased tender joint count were seen in both groups ($P < 0.05$) and a non-significant tendency of decreased swollen joint count was seen in SO group ($P < 0.05$), with no significant group differences. 23 % of patients had elevated values of faecal calprotectin suggestive of nonactive asymptomatic colitis (no values above 110 mg/kg), with no difference between patients using NSAIDs and not ($P = 0.6$).

5.4 Paper IV: The ratio of n-6 to n-3 PUFA was 11 to 1 and 10 to 1 in standard diet and in red blood cells (RBC) of control rats, respectively. Following administration of DSS for 7 days, the ratio in RBC fell in all treatment groups. The lowest ratios were seen in the groups receiving DSS + fish or seal oils (around 6 to 1), but 7 days pretreatment with these oils did not significantly influence the subsequently DSS-induced colitis. In fact, all the oils tended (not significantly) to exacerbate the inflammation. Soy oil increased the mean crypt score.

5.5 Paper V: Different *RF* values were estimated in the entire PGE_2 range studied (0.0125-0.375 ng) at an IS concentration level (0.0125 ng) equivalent to 1/3 of the total PGE_2 calibration range, suggesting that the interaction eicosanoid/IS is an important factor that affects the validity of the *RF* and consequently the accuracy of plasma PGE_2 analysis. By applying a systematic and simultaneous experimental design approach, it is possible to estimate a constant *RF* of 0.075 and perform a reliable eicosanoid quantification using an amount of IS between 0.345 and 0.375 ng.

5.6 Paper VI: Short-term (10 days) duodenal administration of WO or SO reduced ratios of n-6 to n-3 PUFA and AA to EPA and PGE_2 levels in plasma (tendency with WO), with no significant group differences. Correspondingly, joint pain and IBD-disease activity were reduced and QoL was improved in both groups, with no significant group differences.

6. Discussion

6.1 Effects of marine oils in inflammatory bowel disease and psoriatic arthritis

While COX-inhibitory drugs rapidly ameliorate joint pain [27], adverse effects limit their use, particularly in IBD-patients [7], enforcing the need for safe adjuvant or alternative treatments.

In papers I and VI, 10 days duodenal administration of SO or WO both reduced IBD-related joint pain (with prolonged effects of SO in paper I), while IBD-disease activity (index) was slightly reduced, consistent with findings in our pilot study [54]. Faecal calprotectin did not change in patients with moderate IBD-disease activity as shown previously [54, 71], suggesting no adverse effects of oil administrations. IBD-patients generally have poor HRQOL [91], and associated joint pain contributes considerably [6]. SO improved the bodily pain dimension of HRQOL according to the generic SF-36 questionnaire in paper II and both SO and WO improved QoL according to the stomach specific SF-NDI questionnaire in paper VI. In comparison, when given orally for 14 days (10 mL × 3 daily) to patients with IBD-related joint pain, SO did not reduce joint pain or IBD-disease activity compared with cod liver oil [71].

Correspondingly, long-term oral administration of fish oil is necessary for beneficial effects in RA patients [4]. Similarly, 14 days oral administration of SO in paper III did not reduce joint pain or skin affections in PsA patients, while no significant adverse effects of oil administration were seen. The improvement in patients' global assessment of disease four weeks post treatment in SO group was probably due to reduction in symptoms not assessed, e.g. morning stiffness, fatigue or disturbed sleep. These results are in accordance with previous studies of LC n-3 PUFA administration in PsA documenting no effect [56] or delayed effect [55].

6.2 Experimental colitis study

A high dietary ratio of n-6 to n-3 PUFA may be involved in the pathogenesis of IBD, as reviewed [92]. In paper IV, soy oil increased the mean crypt score, a notable early feature of DSS induced damage [93], consistent with its supposedly pro-inflammatory effects due to high LA levels [94]. Pre-treatment with SO or cod liver oil did not protect against subsequent DSS-induced colitis in rats. Indeed both marine oils tended to exacerbate the DSS-induced colitis as seen previously with LC n-3 PUFA [95], possibly in part due to stress associated with gastric gavage feeding as discussed in paper IV. Mixing the oils into the diet would avoid the need for such force feeding. Unfortunately, disease activity index [96] and assessment of rectal bleeding were not included in paper IV.

6.3 Oil dosage and contents

EPA and DHA are presumably the main bioactive components in marine oils [3], giving reason for the use of highly purified LC n-3 PUFA capsules, e.g. free FA or ethyl esters. In the present thesis, natural TAG oils were preferred, as they may contain other beneficial FA and fat soluble vitamins as described later. Interestingly, capsules with LC n-3 PUFA in the form of free FA were not effective in recent EPIC (Epanova Program in Crohn's Study) trials in CD patients [97].

An anti-inflammatory dosage of minimum 2.7 g EPA+DHA daily is moderately effective in long-term treatment of RA, ideally with a concomitant low n-6 PUFA intake [4]. While no dose-response trial was performed, the 30 mL daily oil dosage used in papers I-III, VI and previous studies [54, 71, 98], provided a medium high daily dosage of EPA+DHA (3.1 g for WO and between 4.2 to 5 g for SO). SO and WO also provided a daily dosage of 0.9 to 1.1 g and 0.7 g DPA, respectively. Interestingly, plasma phospholipid DPA was recently found to correlate inversely with CRP in patients with active IBD [99]. SO and WO also provided 0.35 to 0.7 g and 0.6 g stearidonic acid daily, respectively, possibly weighing partially up for the low EPA

content compared with fish oil [100]. Notably, SO and WO provided 11.9 to 14.6 g and 13.1 g sum monounsaturated fatty acids (MUFA) daily, respectively, and oleic acid (18:1n-9) constituted 4 to 4.4 g and 3.4 g per day in SO and WO groups, respectively.

Vitamin D deficiency is common in rheumatic out-patients [101] and IBD-patients [102]. Interestingly, mice lacking vitamin D receptor may develop colitis [103], but vitamin D deficiency is likely to be consequential rather than causal in IBD [92]. Indeed vitamin D supplementation may benefit CD patients [104] and vitamin D drugs are used in psoriasis [53]. Slightly reduced IBD-disease activities (indexes) were seen after duodenal administration of crude SO providing 61 % and soy oil providing 55 % of recommended daily allowance (RDA) vitamin D in paper I and after duodenal administration of refined SO providing 11 % and WO providing 57 % RDA vitamin D in paper VI. However, administration of crude SO or cod liver oil, extremely rich in vitamin D, had no protective effect on colitis (paper VI), nor had refined SO or cod liver oil any effect on IBD-disease activity when given orally in IBD-related joint pain [71]. Unfortunately, serum 25-hydroxyvitamin D (25(OH)D), a biomarker of vitamin D status, was not assessed in the clinical trials.

Indeed we have no direct comparison with refined SO, however crude SO seemed to have a strong effect in paper I. Enforcement of new limits for contaminants in marine oils for human consumption in the summer of 2002 prohibited further use of crude oils in clinical trials. Refining of marine oils may remove beneficial nutrients, antioxidants and other bioactive compounds [51], and particularly cold-pressed WO, i.e. normally not heated above 80°C, may have strong anti-inflammatory effects [42, 44]. However, beneficial effects were still seen with refined SO in the pilot study [54] and paper VI, and with molecularly distilled WO in paper VI.

6.4 Mode of oil administration

It is acknowledged that oral administration of marine oils is a long-term strategy [27], as emphasised in an editorial on paper III. However, many of our patients clearly need

safe analgesics on demand. Although no direct proof of concept concerning the superiority of duodenal over oral administration of marine oils exists, papers I, II and VI and our pilot study [54] suggest that duodenal administration may act as a rapid booster strategy for the relief of joint pain. In a previous study, short-term duodenal administration of SO also reduced stomach pain in patients with subjective food hypersensitivity [98].

Intubation as performed in papers I, II and VI is invasive and uncomfortable, but was astonishingly well accepted in patients with IBD. While duodenal intubation is a useful and not uncommon procedure in patients needing enteral nutrition or endoscopy, rheumatic patients in general are less familiar with the procedure and tube fed marine oils was not as feasible in our PsA patients as in our IBD patients. Duodenal administration is neither feasible for long-term treatment, thus a 10 day study period was considered a compromise between discomfort and benefit. Tube feeding ensures correct dosing and targeted delivery and potential reflux with nausea and vomiting may also be reduced when three proximal GI sphincters are passed [105].

Bolus dosages of 10 mL duodenally administrated marine oils may challenge the digestive and absorptive capacity of the intestine, and possibly thereby influence intestinal mucosal defence. Hydrophobic phospholipids, rich in phoshatidyl-choline (PC), floating on top of the mucous layer, is considered an important mechanism by which mucosal surfaces resist attacks from noxious, water-soluble substances [106]. Interestingly, retarded release PC administration appears effective in the treatment of UC patients [107, 108] and enteric coated capsules with LC n-3 PUFA designed for distal delivery have prophylactic effect in adult [109] and pediatric CD patients [110].

Indeed dietary oils may influence gut microbiota [111] and possibly consequently joint pain [112]. However, the reduced joint pain by marine oils in papers I, II and VI was largely independent of IBD-disease activity consistent with previous studies [54, 71] and SO did not improve gut permeability in our pilot study [54]. Duodenal administration may also strongly stimulate vago-vagal anti-inflammatory reflexes, inhibiting the release of pro-inflammatory cytokines like TNF-

α and interleukin 6, as reviewed [113]. Hence, there are several possibilities of improved effect by duodenal administration of marine oils.

6.5 Fatty acid incorporation and eicosanoid production

Reduced ratios of n-6 to n-3 PUFA and AA to EPA in blood or tissues were seen after marine oil administration in patients with IBD- and PsA-related joint pain (papers I-III and VI) suggesting putatively anti-inflammatory changes.

Tube fed marine oils does not seem to yield a higher incorporation of LC n-3 PUFAs into blood and tissues than corresponding oral administration, but the latter may have effects in PsA (paper III) and possibly IBD-related joint pain [71] given a longer treatment period [27]. A longer pre-treatment with marine oils to rats in paper IV would probably allow a proper fatty acid incorporation into colonic mucosal cell membranes and possibly result in a protective effect on subsequent DSS-induced colitis, as shown previously [3]. Ideally, lipid classes and their fatty acid profiles in blood and tissues ought to be analysed in future studies.

While eicosanoids were not assessed in paper III, LTB_4 levels were previously reduced after Efamol marine administration in PsA patients followed by an increase during placebo administration [56]. Also, a rise in serum thromboxane B_2 (TXB_2) was seen during the placebo period in the Efamol marine group. Recently, 14 days oral administration of SO and cod liver oil both reduced plasma LTB_4 in IBD-patients with joint pain [71]. While LTB_4 may be directly involved in IBD pathogenesis, the role of PGE_2 in IBD is less established, and it may even be protective for the gut mucosa [3]. However, reduced synthesis of nociceptive circulatory PGE_2 levels due to COX-inhibition is considered a potential mechanism by which marine oils as well as COX-inhibitory drugs ameliorate joint pain [4, 24]. Decreased levels of PGE_2 in colonic mucosa [114] and blood mononuclear cells [115] have also been seen after long-term oral administration of fish oil in IBD. In a previous study, administration of 15 mL of a combination of SO and cod liver oil daily for 10 weeks reduced blood PGE_2 levels in healthy volunteers [46].

Paper VI indicates for the first time reduced PGE_2 levels after SO administration alone in IBD-related joint pain and a tendency of reduced PGE_2 levels after WO administration as well, suggesting that COX-inhibition might be a general effect of marine oil administration. As α-tocopherol reduces the release of AA from phospholipids [116], the 30 times lower α-tocopherol level in WO compared with SO in paper VI, together with low EPA content, may explain the non-significant trend of WO on PGE_2 production. The large duodenally administrated dosage and the positional distribution of LC n-3 PUFA on TAG [50] may possibly explain such a rapid reduction in PGE_2 levels. However, as no fish oil group was included in clinical trials, the full impact of *sn*-1 or *sn*-3 versus *sn*-2 positional distribution of LC n-3 PUFA in marine oils on peripheral availability of these fatty acids and their effects upon eicosanoid production remains to be elucidated.

6.6 Oxidative stress

As oxidative stress is increased in chronic inflammatory diseases [117], administrating PUFA rich oils, highly prone to lipid peroxidation, may potentially exaggerate the stress. Olive oil, particularly extra-virgin type, is protective against oxidative stress [118]. While fish oil, SO and WO does not contain as much MUFA, particularly oleic acid, as olive oil, the relatively high content of these FA, known to be very resistant to lipid peroxidation, may possibly be an advantage of marine oils over purified LC n-3 PUFA capsules. Indeed, serum TBARS levels did not increase during oil treatments in IBD-patients or PsA patients (papers I and III), consistent with previous SO studies [54, 71]. In addition to TBARS, it would be advisable to analyse prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), also a biomarker of oxidative stress, in blood or urine [119]. While no recommended limits for TBARS in oils exist, oils did not appear rancid based on smell, taste and appearance. However, including analysis of anisidine and peroxide values in oils, primary and secondary parameters of lipid peroxidation with recommended limits, would be advisable.

6.7 Placebo, blinding and pain issues

Placebo effects are inherent in any clinical intervention, particularly when assessing analgesic effects [120] in uncontrolled studies like our pilot study [54]. When administrating natural TAG oils, finding an adequate placebo is a challenge. While soy oil may not be considered an inert placebo, vegetable oils are widely used in Western diets [1] and was considered in this context an acceptable control oil in papers I-III. Clear differential effects of SO and soy oil given duodenally in a double blind set up in papers I and II, and the unexpected tendency of increased joint pain with soy oil in paper I, prompted the use of SO as "active" control group in paper VI, limiting the use of placebo as recommended in the Helsinki Declaration.

Oil preferences did not predict outcome in paper VI, suggesting that response expectations had minor influence on the relative effects of SO and WO. Whether the characteristic smell of the crude SO and the tendency of adverse effects of soy oil may have contributed to a minor blinding bias in paper I is unknown, as blinding rate was not assessed. When administrating refined marine oils, 37.5 % and 78 % of patients were blinded in papers III and VI, respectively. While the former blinding rate may be acceptable, the higher latter blinding rate may reflect similar appearance, smell and taste of SO and WO. Administrating capsules would possibly ensure enhanced blinding rate, but this was not feasible in our setting.

Of the IBD-patients in papers I and VI respectively, only 10 of 19 (53 %) and four of 18 (22 %) had arthritis. While arthritis is a more severe condition than arthralgia, and consequently possibly more difficult to treat, joint pain is a mutual symptom in IBD, RA and PsA patients. Interestingly, effects of oil administrations seemed equal in patients with and without arthritis in papers I and VI. As pain is importantly the fifth vital sign in clinical medicine [121], joint pain undeniably deserves attention and adequate treatment irrespective of arthritis. While more comprehensive alternatives are being introduced [122], VAS scores are simple but reliable ways of measuring pain [123], widely used together with HRQOL for assessing analgesic drug effects. Joint effects were ascertained by a rheumatologist in

paper I and plasma PGE_2 levels were reduced after oil treatment in paper VI, supporting a real effect of the marine oils.

6.8 Faecal calprotectin levels in psoriatic arthritis patients

At least 15 % of PsA patients have acute or chronic enteric inflammation [21, 22], predominantly those with axial disease. In paper III, as many as 23 % of PsA patients, none with axial disease, had elevated calprotectin levels in stool suggestive of asymptomatic, inactive colitis. Importantly, for confirmation of enteric inflammation, other intestinal measures including endoscopy with histology of intestinal biopsies are required. Interestingly, 44 % of mainly RA patients on NSAIDs had increased calprotectin levels in stool, with high levels also found in healthy volunteers, suggesting NSAID induced enteropathy [124]. However, faecal calprotectin levels did not differ between those taking NSAIDs and not in paper III, though most PsA patients (85 %) used such drugs. In comparison, one IBD-patient (5 %) and three IBD-patients (17 %) used NSAIDs in papers I and VI, respectively. In general, whether NSAIDs or the rheumatic disease is responsible for the enteropathy in SpA like PsA, is uncertain [19, 124]. As recommended in an editorial on paper III, a multidisciplinary approach involving a dermatologist, rheumatologist and gastroenterologist may be recommended for PsA patients [125]. This may possibly also apply for IBD patients having frequent joint and skin affections [12].

6.9 Choice of internal standard amount in eicosanoid quantification

Although numerous articles describe the quantification of PGE_2 using the IS method, few explain how the amount of IS added to the samples was determined. Usually, the amount of IS added to the samples is carried out by trial and error methods or rule of thumb techniques such as targeting the IS to the lower 1/3 of the calibration curve. In addition, despite the fact that the validity of the IS method relies on assumptions of

linearity of the detector response towards both the analyte and IS, information about the latter is commonly unacknowledged. Regardless of the analyte, the concentration ratio analyte/IS could affect the quantitative determination by LC-MS^n [126, 127], but unfortunately such a relationship is generally ignored. NIFES has recently proposed a general experimental design approach for determining a range where the *RF* does not change with the concentrations of analyte and IS [128].

Notably, a fixed level of 0.125 ng IS (corresponding to the lower 1/3 of the PGE_2 calibration curve as recommended by some orthodox approaches) provides seven different *RF* (0.025-0.085) when the amount of PGE_2 is varied between 0.013 and 0.4 ng (Figure 2 in paper V), while a constant *RF* (0.075) is obtained in the previous analytical range (0.013-0.4 ng of PGE_2) when the IS is varied between 0.345 and 0.375 ng. Thus, paper V demonstrates that the application of experimental design enables estimating the interaction PGE_2/IS and to select confidently an optimal amount of IS and a *RF* for analysing eicosanoids in a high number of samples, where the amount of sample is limited and the unknown levels of eicosanoids are spanned in a wide range of concentrations.

6.10 Limitations and future perspectives

A small and slightly heterogeneous IBD-group may have contributed to baseline group differences in papers I and VI. Interestingly, the groups with significantly worse baseline joint status had the lowest intake of fish and marine oils and the lowest tissue levels of LC n-3 PUFA at baseline (significantly lower in paper I). Indeed a higher baseline LC n-3 PUFA status may predict a less effect of LC n-3 PUFA treatment [129]. If feasible, screening patients in advance or allowing a run-in period to normalize baseline levels, as in paper VI, might be advisable.

As vitamins D and E in administered oils may possibly influence on effects measured, it would be advisable to adjust levels and use similar antioxidant. A more extensive background diet assessment might reveal other nutrients of importance for outcome [130]. Ideally, including fish and other seafood in the diet, good sources of

LC n-3 PUFA in addition to other beneficial nutrients like e.g. vitamin D, selenium, iodine, vitamin B_{12}, taurine and well balanced protein may possibly be beneficial for PsA and IBD patients [53, 131]. Besides, as the bioavailability of LC n-3 PUFA may depend on the dietary source [132], research on the impact of fish versus various dietary supplements is advisable.

Mechanisms of effect of SO and WO were only briefly assessed, thus future studies should analyse e.g. cytokines (particularly TNF-α as reviewed [125]), nuclear factor (NF)-kappa B, resolvins [133] and other eicosanoids in specific cells or tissues. In particular, more research on the effects and mechanisms of duodenal versus oral administration of marine oils is necessary. As the clinical studies were explorative pilot studies, effects in IBD-studies and potential delayed effects in PsA study should be confirmed in larger, double blind randomized controlled trials with long-term oral administration of SO, WO and fish oil.

7. Conclusions

7.1 Individual papers

Paper I: As short-term duodenal administrations, SO significantly relieved IBD-related joint pain with prolonged effects compared with soy oil.

Paper II: As short-term duodenal administration, SO normalised n-6 to n-3 FA ratio in rectal biopsies and reduced HRQOL in patients with IBD-related joint pain.

Paper III:
As short-term oral administrations, SO did not improve joint or skin manifestations in PsA compared with soy oil.

Paper IV: Short-term pre-treatment with SO, cod liver oil or soy oil in addition to standard diet did not protect against DSS-induced colitis in rats.

Paper V: The interaction PGE_2/IS is an important factor that affects the validity of the *RF* and consequently the accuracy of the analysis of PGE_2 by LC-MS^n.

Paper VI:
As short-term duodenal administrations, WO and SO appear equally effective in reducing IBD-related joint pain, possibly in part mediated by COX-inhibition, as suggested by reduced PGE_2 levels.

7.2 Overall conclusion

The thesis suggests that short-term duodenal administration of oils from marine mammals, like SO and WO, reduce joint pain and IBD-disease activity and improve QoL without significant adverse effects in IBD-patients with moderate disease activity, while short-term oral administration of SO does not improve joint or skin affections in PsA patients.

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Errata

Paper I:

Correct institutional association of co-authors are NIFES; Institute of Medicine, University of Bergen and Haukeland University Hospital.

Under Methods, MHAQ values should range from 1 (no problem to perform) to 4 (impossible to perform).

In Table II the TBARS-denomination in the seal oil should read WW. The last sentence in the corresponding table text should be changed to "WW = wet weight." Sum vitamin A in the corresponding table text should read sum retinol (13-, 11-, 9-cis and all-trans retinol, i.e. A_1) and 3,4 didehydro-all-trans retinol (A_2).

Under Statistics, sentence 5 should read: "Normality of data was tested with the Kolmogorov-Smirnov test."

The following references should read:

Reference 2: "Holden W, Orchard T, Wordsworth P. Enteropathic arthritis. Rheum Dis Clin North Am 2003;29:513-530, viii."

Reference 5: "Thomas PD, Keat AC, Forbes A, Ciclitira PJ, Nicholls RJ. Extraintestinal manifestations of ulcerative colitis following restorative proctocolectomy. Eur J Gastroenterol Hepatol 1999;11:1001-5.

Reference 15: "Brox J, Olaussen K, Østerud B, Elvevoll EO, Bjørnstad E, Brattebog G, Iversen H. A long-term seal- and cod-liver-oil supplementation in hypercholesterolemic subjects. Lipids 2001;36:7-13."

Reference 16: "Brockerhoff H, Hoyle RJ, Hwang PC, Litchfield C. Positional distribution of fatty acids in depot triglycerides of aquatic animals. Lipids 1968;3:24-9."

Reference 21: “Pincus T, Summey JA, Soraci SA Jr, Wallston KA, Hummon NP. Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 1983;26:1346-53.”

Reference 22: “Harvey RF, Bradshaw JM. A simple index of Crohn’s-disease activity. Lancet 1980;1:514.”

Reference 34: “von Schacky C, Fischer 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;76:1626-31.”

Reference 35: “Kinsella JE. Lipids, membrane receptors, and enzymes: effects of dietary fatty acids. J Parenter Enteral Nutr 1990;14:200S-17S.”

Paper II:

Correct institutional association of co-authors are NIFES; Department of Biomedicine, University of Bergen; Institute of Medicine, University of Bergen and Haukeland University Hospital.

Paper III:

Table 3: Calprotectin concentration should read microgram/L, not mg/L.
Nine of the original 43 patients (21 %) included had elevated levels of faecal calprotectin, and nine of the 40 patients (23 %) finishing the study had elevated levels of faecal calprotectin.

MHAQ refers to modified health assessment questionnaire. MHAQ values range from 1 (no problem to perform) to 4 (impossible to perform). For reference to MHAQ, it is referred to Material and methods chapter.

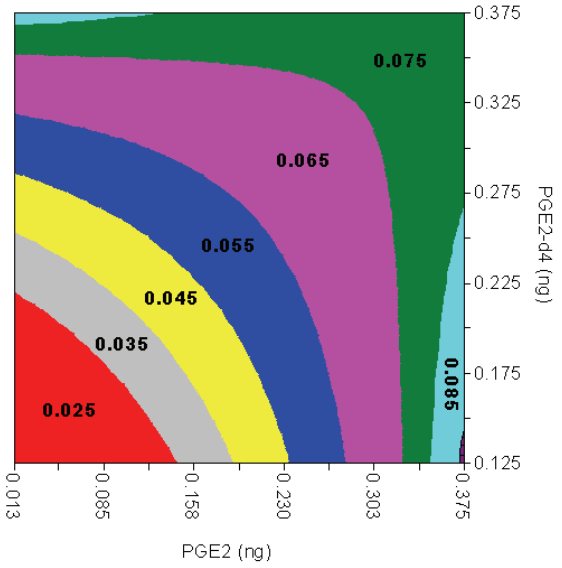
Paper IV:

In the table text of Table 2, sum vitamin A should read sum retinol (13-, 11-, 9-cis and all-trans retinol, i.e. A_1) and 3,4 didehydro-all-trans retinol (A_2).

Paper V:

Correct institutional association of co-authors are NIFES; Department of Biomedicine, University of Bergen; Institute of Medicine, University of Bergen and Haukeland University Hospital.

Figure 2: The correct version is given below.



In addition to the above corrections, which were submitted when delivering the thesis for review, the following changes have been made before final printing:

Page 3: The English version of the logo for Haukeland University Hospital is used.

Page 9/Paper VI: a revised version (including contents, title and original errata) of the submitted manuscript is accepted for publication (in press, www.sciencedirect.com).

Page 13-14: The following abbreviations with explanation were introduced for clarity: ALA, CRP, DPA, ELISA, GLC, GMP, HUH, HPLC, LCITMS, LMF, LOQ, LOX, MHAQ, MUFA, NIFES, PASI, PGE_2-*d4*, SF-36, SF-NDI, SpA, TAG, TBARS, TNF-α, UoB and w/w. Inconsistently used abbreviations are corrected throughout the thesis.

Page 23: "Leukotriene (LTB_4) is changed to " leukotriene B_4 (LTB_4)".

Page 25: "43 %" is changed to "43 % (34 patients)"

Page 29: Table 1; "n-3" is changed to "LC n-3 PUFA" with abbreviation explained in table text. Besides, "VO" in table text is clarified as "vegetable oil (soy oil)".

Page 30: "WO" is changed to "WO (from adult minke whales)".

Page 35: "Vitamin D_2" is changed to "vitamin D_2 (ergocalciferol) and "vitamin D_3" is changed to "vitamin D_3 (cholecalciferol)".

Page 37: "ANOVA" is changed to "ANOVA (analysis of variance)".

Page 41: "EPIC" is changed to "EPIC (Epanova Program in Crohn's Study)".

Page 42: "25 (OH)D" is changed to "25-hydroxyvitamin D (25(OH)D)".

References: Due to a mishap with the reference system, some of the references have been corrected, notably number 50, 58, 63-65, 67, 69, 73, 75, 86, 88-90, 94, 97, 112 and 114 (some of these references have been used in papers).

A few linguistic and layout errors have been corrected throughout the thesis (resulting in no scientific changes): e.g. "prostaglandin E2" is changed to "prostaglandin E_2".