Systematic Review

Polyunsaturated Fatty Acids and Chronic Pain: A Systematic Review and Meta-analysis

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Background: Chronic pain is one of the most frequent disease symptoms and represents a global health problem with a considerable economic burden. The role of polyunsaturated fatty acids (PUFA) in chronic pain conditions was debated during the last decade with conflicting results.

Objective: To assess whether polyunsaturated fatty acids intake is useful as a preventive or curative tool in chronic pain.

Study Design: Systematic review and meta-analysis.

Setting: This study examined all published studies, either preventive or curative, on PUFA supplementation and chronic pain.

Methods: We retrieved studies published in any language by searching systematically Medline, Embase, Conference Proceedings Citation Index, dissertations databases, and the 5 regional bibliographic databases of the World Health Organization until May 2015.

We included both observational and intervention studies reporting effect measures and their confidence intervals of polyunsaturated fatty acids intake in the regular diet or supplementation and pain.

Two investigators selected studies; extracted data independently on baseline characteristics, exposure, and outcomes; and rated the quality of interventional studies using Jadad score.
We calculated pooled standardized mean differences (SMDs) of pain indexes such as the Visual Analogue Score. We further carried out subgroup analyses by disease, type of PUFA, outcome scale, quality index, dose, and time of supplementation.

Results: We retrieved 5 observational and 46 intervention studies. Only one observational study showed a protective effect of PUFA. On the contrary, the interventional studies yielded a pooled random effects SMD of -0.40 (95% CI -0.58, -0.22), which indicates improvement, as 0 is the value that indicates absence of effect. The largest effect was found for dysmenorrhea (SMD -0.82, 95% CI -1.21, -0.43), Ω-3 supplementation (-0.47, 95% CI -0.68, -0.26) and composite scores (-0.58, 95% CI -1.07, -0.09). Mitigation of pain was stronger for low doses (-0.55, 95% CI -0.79, -0.30) and short supplementation periods (-0.56, 95% CI -0.86, -0.25).

Limitations: While the number of curative studies was large, that of preventive studies available was limited.

Conclusion: Our results suggest that Ω-3 PUFA supplementation moderately improves chronic pain, mainly that due to dysmenorrhea. Further investigation on the preventive potential of PUFA supplementation is needed, as the amount of evidence is scarce.

Key words: Meta-analysis, systematic review, chronic pain, PUFA, supplementation, Ω-3, dysmenorrhea

Protocol registration: PROSPERO nº CRD42014010064

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Chronic pain represents an important global health problem. Its prevalence worldwide varies between 10% and 25%, and one-tenth of the adult population is diagnosed with chronic pain every year with a median duration of 7 years per episode (1). There is strong evidence that the contribution of chronic pain to the global burden of disease was, so far, underestimated. The World Health Organization (WHO) predicts that by 2030 the main contributors to the global burden of disease will be unipolar depression, coronary heart disease, cerebrovascular disease, and traffic accidents, all of which are associated with chronic pain as co-morbidity (2).

Furthermore, chronic pain implies a high cost which reaches $635 billion annually in the USA and €200 billion in Europe (3), higher than that of cancer and cardiovascular diseases together (4).

The role of polyunsaturated fatty acids (PUFA) as an effective treatment for chronic pain conditions, such as rheumatoid arthritis and osteoarthritis, was debated in the last decade (1,5,6). Ω-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) decrease the activity of Ω-6 PUFA arachidonic acid (ARA), a fatty acid that is related to the production of inflammatory mediators (7). So far, the results are conflicting, both in intervention and prevention studies, and range from improvement to absence of effect or even worsening of pain-producing conditions (8-11). Previous reviews, which used proxy measures of pain, have commented on the association between fatty acids intake and specific diseases in particular settings (5,6,12). However, no comprehensive meta-analysis was carried out so far. We therefore summarized the scientific evidence through a systematic review and meta-analysis of the effect of PUFA's on pain syndromes.

Methods

We followed the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines (13). The review protocol was registered in PROSPERO (14).

Search Strategy

We searched MEDLINE, EMBASE, the 5 regional bibliographic databases of the World Health Organization (WHO) (African Index Medicus, Latin American and Caribbean Health Science Literature Database, Index Medicus for the Eastern Mediterranean Region, Index Medicus for South-East Asia Region, Western Pacific Region Index Medicus, the Conference Proceedings Citation Index database, and the Open Access Thesis and Dissertations (OATD), from inception to May 2015. We used the algorithm, both in free text and Medical Subject Headings: PAIN AND (FATTY ACIDS OR OMEGA-3 OR PUFA) AND (CLINICAL TRIALS OR CASE-CONTROL OR COHORT) as the main search. Additionally, we performed searches with the key words in free text "abdominal pain," "low back pain," "joint pain," "headache"; and "fatty acids" to ensure that every study was retrieved. These key words were selected according to the most prevalent pain syndromes cited in the literature (15). We also reviewed the references of every article retrieved and those of recent reviews and monographs (7,16).We did not include any language limitations. All searches were carried out independently by 2 researchers and the results were merged.

Inclusion Criteria and Data Collection

Studies were included if (1) they presented original data from observational or intervention studies, (2) the outcome was clearly defined as chronic pain, according to the definition of the International Association for the Study of Pain (17), (3) pain outcomes were measured through validated tools such as visual analogue scales (VAS) or composite scores for specific pain syndromes, (4) exposure was PUFA intake or supplementation, (5) they presented effect measures (means, mean difference, mean change) with their confidence intervals (CI), standard error, or enough data to calculate them (P-value, raw data, interquartile range).

We excluded studies on childhood pain and acute pain, and also those that did not specify the type of fatty acid. If data were duplicated in more than one study, the most recent one was used. When an article reported results for more than one pain-PUFA relationship (i.e., different supplements), we considered each one as a separate study unit. When necessary, we contacted authors for further information (9,10,18). The studies retrieved were divided into 2 groups, according to their preventive or curative purpose.

We developed a structured questionnaire to extract the following data: (1) author, (2) year of publication, (3) country, (4) study design, (5) sample size, (6) disease, (7) exposure (intervention group and comparison group), (8) pain measurement instrument, (9) effect measure, and (10) adjustment factors for observational studies. Additionally, for clinical trials, we extracted: (11) intervention period and (12) study duration.

Quality Assessment

The quality of clinical trials was assessed using the Jadad index which rates aspects of randomization, blinding, and withdrawals (19). A score of 3 or higher out of 5 was considered as "low risk of bias."

Both the extraction process and the quality rating were performed by 2 researchers independently, and results were merged by consensus. Due to the small number of the studies retrieved (5 with 3 different designs) and thus, the impossibility of performing a meta-analysis, we did not assess the quality of observational studies through a standard scale.

Data Synthesis and Analysis

Study-specific standardized mean differences (SMDs) were weighted by the inverse of their variance to compute a pooled effect size and its 95% CI.

SMDs are used as summary statistics when all the studies assess the same outcome but measure it in a variety of ways (as in the case of different measurement scores). SMDs compare treatment and placebo and are weighted by the pooled standard deviation (SD) of both arms. An SMD of zero means no difference between treatment and placebo, a negative result favors treatment in our case (as the pain score decreases when treatment is effective) and a positive result favors placebo. A large effect is defined as > 0.8 unit, a medium effect as > 0.5 unit, and a small effect as < 0.2 units (20). When all the information required was not available, we assumed normal distribution of SMDs and used median and interquartile range to calculate means and SDs. Furthermore, when a study reported different doses of the same supplement, or included pain from different anatomical sites (e.g., joint and abdominal pain), we first pooled its various SMDs and included this data in the main analysis. We later stratified our results by subgroup.

We assessed heterogeneity using Cochran's Q-statistics and the proportion of the total variance due to between-study variance I2. We present both fixed effects and random effects pooled estimates, but used the latter when heterogeneity was present.

Cumulative Meta-analysis

In addition to the general meta-analysis, we performed a cumulative meta-analysis by date of publication both for all pain outcomes and for rheumatoid arthritis. The objective was to detect a possible trend in the estimation.

Subgroup Analyses

In addition to the main analysis of studies of fatty acid supplementation and chronic pain, we carried out subgroup analyses by disease (rheumatoid arthritis, dysmenorrhoea, mastalgia, other chronic pain syndromes), type of fatty acid supplementation (Ω-3, Ω-6, dietary intervention/supplement, combined), outcome scale (VAS/Likert scale, composite score), and Jadad score (low, high).

Further subgroup analysis includes studies administering Ω-3 supplementation up to 3 months and longer than 3 months. These intervals were based on previous reports suggesting that the therapeutic effects of Ω-3 PUFAs are observed after approximately 3 months (5). We also stratified by daily dose of EPA (< 1.35 and > 1.35 g/day). We chose this threshold because the effect on pain was observed in the interval 1.35 – 2.7 g/day in previous studies (21). When doses were given in mg/kg/day, we calculated the total daily dose for an adult of 70 kg.

All subgroups analyses were planned *a priori*.

Publication Bias

We used funnel plots to detect publication bias and, more formally, Egger's regression test (22), and the Trim-and-Fill method to correct for potential publication bias (23).

Statistical analysis was performed with Comprehensive Meta-analysis software (Biostat, Englewood, NJ).

Results

Our search brought 47 articles which provided data on 51 studies: 5 observational studies (10,11,24-26) and 46 intervention studies (8,9,18,27-65). The complete search strategy and exclusion motives are shown in Fig. 1. The main characteristics of the studies included are shown in Table 1. We performed separate analyses for observational studies and intervention studies. As the number of preventive studies was scarce and different designs were used, we were unable to carry out a meta-analysis. Therefore, in this part, we limited our study to a narrative review.

Observational Studies

We found 5 observational studies (3 cross-sectional, one case-control, and one cohort study), carried out in 4 countries, that met our inclusion criteria. The data from one study were obtained from the authors (10).

In Denmark in 1995, Deutch (11) carried out a

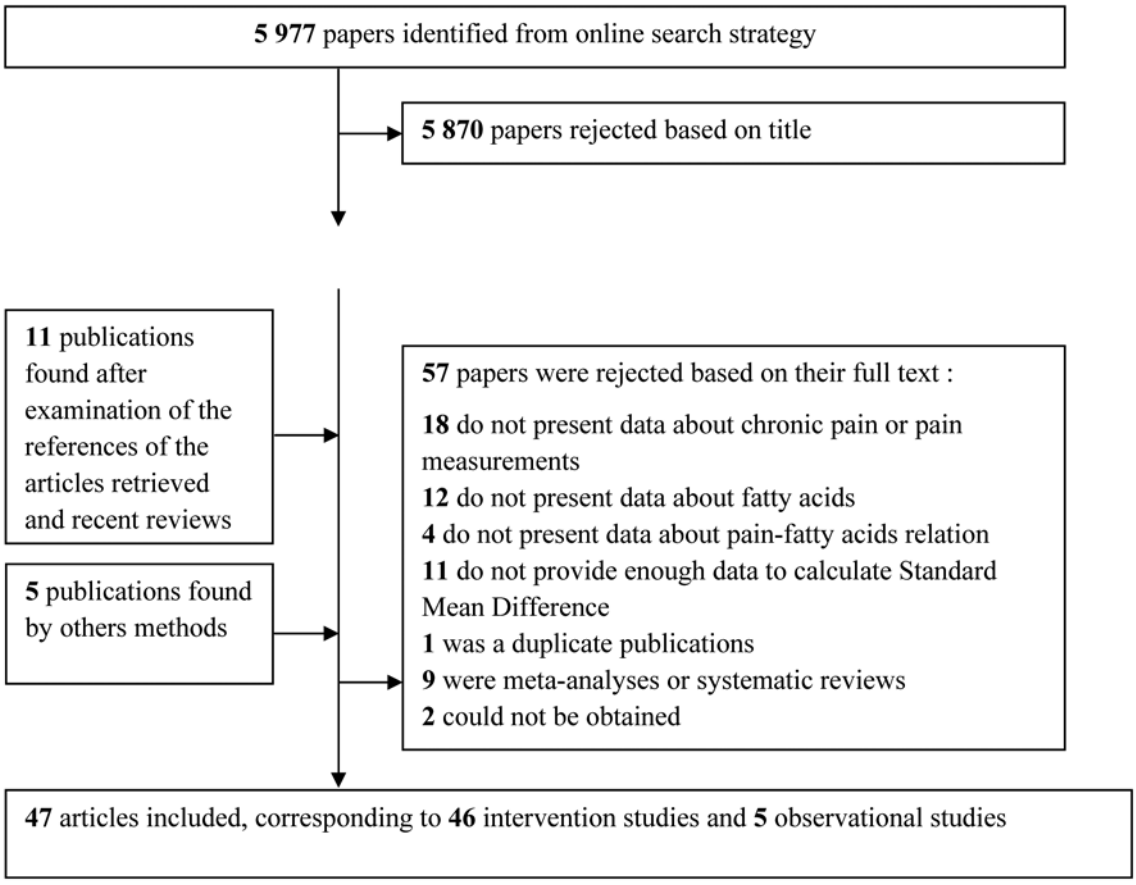

Fig. 1. *Flow diagram of search strategy according to the PRISMA guidelines.*

cross-sectional study among 181 healthy volunteers about menstrual pain and dietary habits using a self-administered questionnaire distributed at the beginning of the study and 6 months later. The author reported a strong association between increased pain and a low intake of Ω-3 fatty acids from fish or fish oils (r = 0.53) and between increased pain and a low ratio of Ω-3 to Ω-6 fatty acids in the diet (r = 0.42).

Two other cross-sectional studies were carried out by Nagata et al in Japan in 2004 and 2005 (24,25) among nursing students on dietary habits and premenstrual syndrome and menstrual pain, with 189 and 276 participants, respectively. In both studies no association was found between PUFA intake and any of the syndromes.

In 1997, Di Cintio et al (26) carried out a case-control study in Italy on dietary habits, reproductive and menstrual factors, and risk of dysmenorrhoea. Dietary habits were assessed by trained interviewers using a standard questionnaire in 106 cases and 145 controls recruited at an outpatient gynecological clinic. These authors did not find any association between Ω-3 or Ω-6 intake and dysmenorrhoea.

Finally, in 2011 a Spanish cohort study on dietary habits and quality of life, with 4 years of follow-up and 8,430 participants showed no association between PUFA intake and bodily pain or other SF-36 questionnaire domains related to pain (10). To summarize, only one cross-sectional study out of these 5 observational studies found a protective effect of fatty acids on pain.

Intervention Studies

We found 46 studies (45 clinical trials and one quasi-experimental study) carried out in 18 different countries. The majority of the studies was on rheuma-

Table 1. *Main characteristics of the studies.*

Observational Studies									
Study (year)	**Country**	**Design**	**Disease**	**Population**	**Sample size**	**Exposure**	**Measure time**	**Outcome score**	**Impairment/ Adjust/Restriction factors**
Ruano et al (2011)	Spain	Cohort	Bodily pain	University	15,089 entered, 8,430 included	Dietary fatty acid intake (saturated and trans-unsaturated fats, MUFAs and PUFAs)	Twice (0 - 4 years)	SF-36 Health Survey	Age, sex, smoking, leisure time physical activity, total energy intake, BMI, Mediterranean Diet Score
Nagata et al (2005a)	Japan	Cross-sectional	Premenstrual syndrome	University – nursing school	315 entered, 189 included	Dietary fatty acid intake (saturated fats, MUFAs and PUFAs)	Once	MDQ score	Age, total energy intake, marital status, smoking status, exercise, age at menarche, number of days of bleeding
Nagata et al (2005b)	Japan	Cross-sectional	Menstrual pain	University – nursing school	362 entered, 276 included	Dietary fatty acid intake (saturated fats, MUFAs and PUFAs)	Once	Verbal multidimensional scoring system	Age, total energy intake, smoking status, age at menarche
Di Cintio et al (1997)	Italy	Case-control study	Dysmenorrhea	Outpatient clinic	106 cases and 145 controls	Nutritional habits (whole foods, fats and condiments consumption)	Once	Andersch and Milsom's classification, VAS	Age, education, smoking, selected menstrual characteristics, reproductive history
Deutch (1995)	Denmark	Cross-sectional cohort	Menstrual pain	Community based - volunteers	44 cases and 137 controls	Dietary fatty acid intake (saturated fats and PUFAs)	Twice (0 - 6 months)	Self-administered questionnaire	Parity, time of food record, total energy intake.

Intervention Studies									
Study (year)	**Country**	**Intervention period**	**Disease**	**Sample size (Treatment/ Control)**	**Intervention**	**Daily dose**	**Comparison**	**Outcome score**	**Standard Mean Difference (95% CI)**
Lopes Cortes (2013)	Brazil	12 weeks	Myofascial pain syndrome	41 (20/21)	Ω-3	1080 mg EPA, 720 mg DHA	_	VAS	-0.87 (-1.38, -0.35)
Park et al (2013)	Korea	16 weeks	Rheumatoid arthritis	109 (41/40)	Ω-3	2090 mg EPA, 1160 mg DHA	Sunflower oil	VAS	0.30 (-0.14, 0.74)
Sohrabi et al (2013)	Iran	3 months	Premenstrual syndrome	139 (63/61)	Ω-3 (1m), 8 days before and 2 after menstruation (2m)	360 mg EPA, 240 mg DHA	Placebo	VAS	-0.41 (-0.76, -0.05)
Ramsden et al (2013)	USA	12 weeks	Chronic headaches	67 (33/34)	Dietary High Ω-3 + low Ω-6 intervention	_	Low Ω-6	HIT-6	-0.94 (-1.45, -0.44)

Table 1. *Main characteristics of the studies (continued).*

Intervention Studies									
Study (year)	**Country**	**Intervention period**	**Disease**	**Sample size (Treatment/ Control)**	**Intervention**	**Daily dose**	**Comparison**	**Outcome score**	**Standard Mean Difference (95% CI)**
Rahbar et al (2012)	Iran	3 months	Primary dysmenorrhea	100 (95/95)	Ω-3	180 mg EPA, 120 mg DHA	Placebo	Cox menstrual symptom scale	-1.22 (-1.53, -0.91)
Caturla et al (2011)	Spain	9 weeks	Osteoarthritis / Reumatoid arthritis	45 (19/12)	Ω-3	222 mg EPA, 178 mg DHA (5 wk) and 111 mg EPA, 89 DHA (4 wk	Placebo	WOMAC score	-1.43 (-2.24, -0.63)
Guimarães et al (2011)	Brazil	28 days	Centrally mediated chronic myalgia	24 (12/12)	Splint + fish oil	3g (no EPA/DHA doses given)	Splint + avoid foods rich in Ω-3	VAS	0.32 (-0.49, 1.12)
Kawabata et al (2011)	Japan	4 weeks	Asthenopia (eye-pain, low back pain, headache)	22 (11/9)	Fish oil	162 mg EPA, 783 mg DHA	Middle chain triglycerides (edible oil)	VAS	-0.75 (-1.66, 0.16)
Moghadamnia et al (2010)	Iran	3 months	Dysmenorrhea	36 (36/36)	Fish oil	550 mg EPA, 205 mg DHA	Vitamins emulsion	VAS	-0.81 (-1.29, -0.33)
Pruthi et al (2010)	USA	6 months	Mastalgia	43 (21/22)	EPO + corn oil	315 mg GLA	Corn oil	VAS	0.18 (-0.41, 0.78)
Das Gupta et al (2009)	Bangladesh	12 weeks	Rheumatoid arthritis	100 (40/41)	Indomethacin + Ω-3	540 mg EPA, 360 mg DHA	Indomethacin	VAS	-1.07 (-1.54, -0.60)
Gruenwald et al (2009)	Germany	26 weeks	Osteoarthritis	177 (90/87)	Glucosamine sulfate + Fish oil + Ω-3	1332 mg Fish oil, 600 mg Ω-3 (no EPA/ DHA dose given)	Glucosamine sulfate + mixture oils	WOMAC score	-0.26 (-0.56, 0.03)
Aryaeian et al (2009)	Iran	12 weeks	Rheumatoid arthritis	52 (22/22)	Conjugated linoleic acid	2500 mg	High oleic sunflower oil	VAS	1.41 (0.7 , 2.07)
Galarraga et al (2008)	United Kingdom	9 months	Rheumatoid arthritis	97 (49/48)	Cod liver oil + fish oil	1500 mg EPA, 700 mg DHA	Identical air-filled placebo	VAS	-0.32 (-0.72, 0.08)
Goyal et al (2005)	United Kingdom	12 months	Mastalgia	278 (140/138)	EPO	320 mg GLA	Coconut oil	Breast Pain Score	-0.03 (-0.27, 0.20)
Berbert et al (2005)	Brazil	24 weeks	Rheumatoid arthritis	55 (17/13)	Fish oil Ω-3 + olive oil	1800 mg EPA, 1200 mg DHA, 6.8g OA	Soy oil	5-point score	-1.32 (-2.12, -0.53)
				55 (13/13)	Fish oil Ω-3	1800 mg EPA, 1200 mg DHA			-0.60 (-1.38, 0.19)
Sundrarjun et al (2004)	Thailand	12 weeks	Rheumatoid arthritis	46 (23/23)	Low Ω-6 diet + fish oil	1880 mg EPA, 1480 mg DHA	Low Ω-6 diet + Placebo	VAS	0.24 (-0.34, 0.82)

Table 1. *Main characteristics of the studies (continued).*

Intervention Studies									
Study (year)	**Country**	**Intervention period**	**Disease**	**Sample size (Treatment/ Control)**	**Intervention**	**Daily dose**	**Comparison**	**Outcome score**	**Standard Mean Difference (95% CI)**
Remans et al (2004)	Netherlands	4 months	Rheumatoid arthritis	66 (26/29)	PUFA supplement drink	1400 mg EPA, 200 mg DHA - 500 mg GLA	Placebo drink	VAS	0.51 (-0.02, 1.05)
Sampalis et al (2003)	Canada	3 months	Premenstrual syndrome and dysmenorrhea	70 (36/34)	KO daily (1m) and 8 days before and 2 after menstruation (2m)	284 mg EPA, 170 mg DHA	Ω-3 fish oil	VAS	-0.82 (-1.31, -0.33)
Guerra dos Santos (2003)	Brazil	3 months	Mastalgia	111 (56/55)	Borage oil	326 mg LA, 180 mg GLA	Sunflower oil	Cardiff Breast Score	0.11 (-0.26, 0.49)
Blommers et al (2002)	Netherlands	6 months	Mastalgia	90 (30/30)	EPO + corn oil + wheat-germ oil	288 mg GLA, 2136 mg LA	Corn oil + wheat-germ oil	4-point score	0.05 (-0.45, 0.56)
				90 (30/30)	Fish oil + corn oil	1128 mg EPA, 714 mg DHA			0.00 (-0.51, 0.51)
Adam et al (2002)	Germany	3 months	Rheumatoid arthritis	60 (30/30)	Fish oil + anti-inflammatory diet	128.1 mg EPA, 95.8 mg DHA / 10 kg of body weight	Corn oil	VAS	-1.92 (-2.54, -1.31)
				60 (30/30)	Fish oil + standard western diet	128.1 mg EPA, 95.8 mg DHA / 10 kg of body weight			-0.96 (-1.50, -0.43)
Harel et al (2002)	USA	2 months	Migraines	27 (23/23)	Marine Ω-3 ethyl ester concentrate	756 mg EPA, 498 mg DHA	Olive oil ethyl ester concentrate	7-point score	-1.32 (-1.96, -0.69)
Volker et al (2000)	Australia	15 weeks	Rheumatoid arthritis	50 (13/13)	Fish oil (60% Ω-3)	40 mg/ kg/d of fish oil (50% EPA, 35% DHA)	50/50 corn/ olive oil	VAS	-0.01 (-0.78, 0.76)
Sarzi-Puttini et al (2000)	Italy	24 weeks	Rheumatoid arthritis	50 (22/21)	Diet rich in unsaturated fat + hipoallergenic foods	2:1 ratio of unsaturated to saturated fat	Normal diet	VAS	-0.16 (-0.76, 0.44)
Hansen et al (1996)	Denmark	6 months	Rheumatoid arthritis	109 (36/45)	Adjusted Graastener diet (800 g fresh fish a week OR fish oil supplement)	600 mg EPA, 420 mg DHA	Normal diet	VAS	-0.37 (-0.81, 0.08)
Zurier et al (1996)	USA	12 months	Rheumatoid arthritis	56 (22/19)	Borage seed oil	2800 mg GLA	Sunflower seed oil	VAS	-0.96 (-1.61, -0.31)
Kremer et al (1995)	USA	48 weeks	Rheumatoid arthritis	66 (15/14)	Diclofenac + fish oil + corn oil	130 mg/ kg/d of Ω-3 (44% EPA - 24% DHA)	Diclofenac + Corn oil	5-point score	0.14 (-0.59, 0.87)
Nordstrom et al (1995)	Finland	3 months	Rheumatoid arthritis	22 (11/11)	Flaxseed oil	9600 mg α-LNA	Safflower oil	VAS	-0.21 (-1.05, 0.62)

Table 1. *Main characteristics of the studies (continued).*

Intervention Studies									
Study (year)	**Country**	**Intervention period**	**Disease**	**Sample size (Treatment/ Control)**	**Intervention**	**Daily dose**	**Comparison**	**Outcome score**	**Standard Mean Difference (95% CI)**
Geusens et al (1994)	Belgium	12 months	Rheumatoid arthritis	90 (21/20)	Fish oil + olive oil	840 mg EPA, 180 mg DHA	Olive oil	5-point score	-1.10 (-1.76, -0.44)
				90 (19/20)	Fish oil	1680 mg EPA, 360 mg DHA			-1.89 (-2.65, -1.14)
Leventhal et al (1994)	USA	24 weeks	Rheumatoid arthritis	34 (7/7)	Blackcurrant seed oil	1995 mg GLA	Soybean oil	VAS	-0.85 (-1.94, 0.24)
Leventhal et al (1993)	USA	24 weeks	Rheumatoid arthritis	37 (14/13)	Borage seed oil	1400 mg GLA	Cotton seed oil	VAS	-1.11 (-1.92, -0.30)
Magaró et al (1992)	Italy	45 days	Rheumatoid arthritis	20 (10/10)	Diclofenac + Ω-3	1600 mg EPA, 1100 mg DHA	Diclofenac	VAS	0.42 (-0.46, 1.31)
Nielsen et al (1992)	Denmark	12 weeks	Rheumatoid arthritis - visual pain	57 (27/24)	Fish oil	2000 mg EPA, 1200 mg DHA	Fat composition ; average Danish diet	VAS	-0.58 (-1.14, -0.02)
Sköldstam et al (1992)	Sweden	6 months	Rheumatoid arthritis	46 (22/21)	Fish oil	1800 mg EPA, 1200 mg DHA	Inactive oil (maize, olive and peppermint oils)	4-point score	0.04 (-0.55, 0.64)
Stammers et al (1992)	United Kingdom	6 months	Osteoarthritis	86 (29/29)	Current NSAIDs + cod liver oil	786 mg EPA	Current NSAIDs + olive oil	VAS	0.21 (-0.31, 0.73)
Brzeski et al (1991)	United Kingdom	6 months	Rheumatoid arthritis	40 (13/17)	EPO	540 mg GLA	Olive oil	VAS	0.15 (-0.57, 0.88)
Kremer et al (1990)	USA	24 weeks	Rheumatoid arthritis	64 (20/12)	Fish oil	27 and 18 mg/kg/day of EPA and DHA	Olive oil	5-point score	0.23 (-0.42, 0.88)
				64 (17/12)		54 and 36 mg/kg/day of EPA and DHA			
Van der Tempel et al (1990)	Netherlands	3 months	Rheumatoid arthritis	16 (14/14)	Fractionated fish oil	2040 mg EPA, 1320 mg DHA	Fractionated coconut oil	VAS	-0.58 (-1.33, 0.18)
Jäntti et al (1989)	Finland	12 weeks	Rheumatoid arthritis	20 (9/9)	20mL EPO (9% GLA)	1800 mg GLA	Olive oil	VAS	0.25 (-0.68, 1.17)
Cleland et al (1988)	Australia	16 weeks	Rheumatoid arthritis	60 (23/23)	Fish oil	3200 mg EPA, 2000 mg DHA	Olive oil	VAS	-0.02 (-0.60, 0.56)
Kremer et al (1987)	USA	14 weeks	Rheumatoid arthritis	40 (33/33)	Fish oil	2700 mg EPA, 1800 mg DHA	Placebo	5-point score	-0.34 (-0.82, 0.15)

EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, GLA: Gamma-linolenic acid

Table 2. *Meta-analysis of intervention studies in chronic pain.*

		Nº of studies	SMD Fixed effects [95% CI]	SMD Random effects [95% CI]	Heterogeneity I2	Q-test *P*-value
Main analysis		46	-0.37 [-0.45 , -0.30]	-0.40 [-0.58 , -0.22]	80.53	0.001
Subgroup						
Disease	Rheumatoid arthritis	29	-0.34 [-0.46, -0.23]	-0,36 [-0.62, -0.10]	80.06	0.001
	Mastalgia	5	0.03 [-0.14, 0.19]	0.03 [-0.14, 0.19]	0.00	0.94
	Dysmenorrhoea	4	-0.85 [-1.04 , -0.66]	-0.82 [-1.21, -0.43]	73.72	0.01
	Others diseases	8	-0.51 [-0.69, -0.32]	-0.61 [-1.03, -0.20]	77.03	0.001
Fatty acid type	Ω-3 (from fish oil)	27	-0.49 [-0.59, -0.39]	-0.47 [-0.68, -0.26]	76.50	0.001
	Gammalinolenic acid	9	-0.08 [-0.24, 0.08]	-0.16 [-0.44, 0.12]	56.19	0.02
	Combined PUFA	3	-0.39 [-0.76, -0.02]	-0.61 [-1.83, 0.60]	90.32	0.001
	Dietary intervention	5	-0.60 [-0.84, -0.36]	-0.63 [-1.30, 0.05]	87.06	0.001
Daily dose (overall)	≤ 1.35g	22	-0.47 [-0.56, -0.37]	-0.55 [-0.79, -0.30]	83.40	0.001
	> 1.35g	20	-0.23 [-0.37, -0.09]	-0.29 [-0.56, -0.03]	69.90	0.001
Daily dose (Ω-3)	≤ 1.35g	17	-0.70 [-0.81, -0.58]	-0.74 [-1.01, -0.48]	79.30	0.001
	> 1.35g	16	-0.16 [-0.31, -0.01]	-0.21 [-0.49, 0.07]	70.40	0.001
Intervention period (overall)	≤ 3 months	21	-0.64 [-0.75, -0.52]	-0.56 [-0.86, -0.25]	84.02	0.001
	> 3 months	25	-0.18 [-0.28, -0.07]	-0.24 [-0.43, -0.06]	66.56	0.001
Intervention period (Ω-3)	≤ 3 months	17	-0.83 [-0.96, -0.70]	-0.78 [-1.04, -0.52]	73.06	0.001
	> 3 months	18	-0.20 [-0.32, -0.07]	-0.24 [-0.47, -0.01]	69.15	0.001
Outcome score	Composite score	6	-0.41 [-0.54, -0.27]	-0.58 [-1.07, -0.09]	90.92	0.001
	VAS and Likert score	40	-0.36 [-0.45, -0.27]	-0.37 [-0.57, -0.17]	77.81	0.001
Jadad score	< 3	10	-0.45 [-0.64, -0.27]	-0.38 [-0.73, -0.03]	68.32	0.001
	≥ 3	36	-0.36 [-0.44, -0.27]	-0.41 [-0.62, -0.20]	82.66	0.001

toid arthritis, used Ω-3 as an intervention, VAS as assessment score for pain, and had low risk of bias according to Jadad score. We retrieved 29 studies on rheumatoid arthritis, 5 studies on mastalgia, 4 studies on dysmenorrhea, and 8 studies on other diseases (osteoarthritis, migraines, asthenopia, chronic myalgia, myofascial pain syndrome). The data from one study were obtained from the authors (9). The main characteristics of the intervention studies are shown in Table 1.

PUFA supplementation was associated with a meaningful reduction in the risk of chronic pain (random effects pooled SMD = -0.40, 95% CI -0.58 to -0.22).

Subgroup analyses (Table 2 and Fig. 2) show that supplementation of PUFA decreased the risk of all painful diseases except mastalgia. The strongest effect was observed for the 4 studies of dysmenorrhoea (SMD -0.82, 95% CI -1.21 to -0.43). The "other diseases" group showed moderate effect (SMD -0.61, 95% CI -1.03 to -0.20), and the rheumatoid arthritis group small to moderate effect (SMD -0.36, 95% CI -0.62 to, -0.10).

Heterogeneity was substantial overall and similarly high after stratification by design, quality features (including adjustment for confounders), and study population. Given the substantial heterogeneity, we focused on the random effects analysis. The fixed effects results are presented for comparison and discussed only where they differ.

When we stratified our analysis by type of fatty acid supplementation, the only subgroup that shows an association with pain is the Ω-3 supplementation (SMD -0.47, 95% CI -0.68 to -0.26). The "combined PUFA" and "dietary intervention" groups show stronger effects, although not statistically significant using the random effects model but significant under the fixed effects model. The Ω-6 group showed no effect.

For the analysis of the Ω-3 group, we excluded one study which used conjugated linoleic acid (8), as it is a precursor and not strictly a Ω-3PUFA. The results after including this study did not change meaningfully (SMD -0.46, 95% CI -0.67 to -0.25).

The stratification by daily dose of fatty acids and duration of the supplementation provided interesting

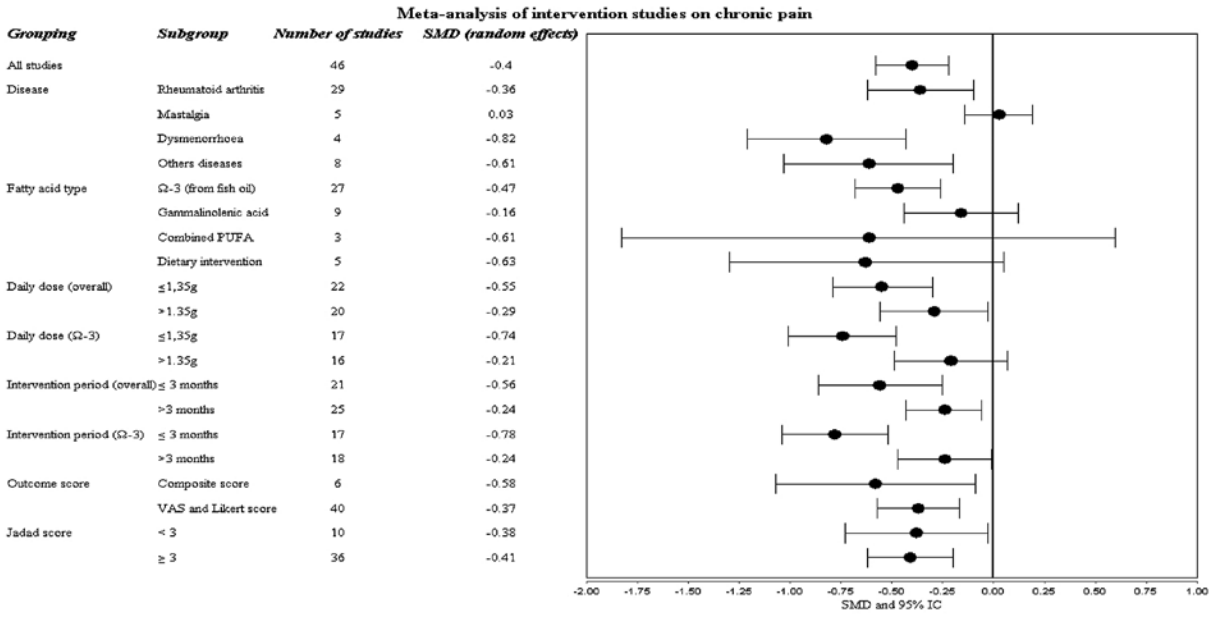

Fig. 2. *Forest plot of random effects subgroup analysis.*

information. Compared to placebo, we observed a stronger effect for the low dose group, i.e., daily intake ≤ 1.35g (SMD -0.55, 95% CI -0.79 to -0.30) than for the high dose group i.e., daily intake > 1.35g (SMD -0.29, 95% CI -0.56 to -0.03). As for the intervention period, we found a larger effect for the ≤ 3 months group (SMD -0.56, 95% CI -0.86 to -0.25) than for the > 3 months group (SMD -0.24, 95% CI -0.43 to -0.06).

Furthermore, when we restricted this dose-response analysis to Ω-3 intervention, again, the effect was larger for the low dose group (SMD -0.74, 95% CI -1.01 to -0.48) than for the high dose group, in which no effect was observed (SMD -0.21, 95% CI -0.49 to 0.07). As in the general analysis, the effect was stronger for short interventions (SMD -0.78, 95% CI -1.04 to -0.52) than for longer ones (> 3 months) (SMD -0.24, 95% CI -0.47 to -0.01).

Studies that used composite scores showed a stronger effect than studies that used VAS or Likert scores (SMD -0.58, 95% CI -1.07 to -0.09 and -0.37, 95% CI -0.57 to -0.17, respectively). No substantial difference was observed when we stratified the analysis by quality score (SMD -0.38, 95% CI -0.73 to -0.03 for low quality studies and -0.41, 95% CI -0.62 to -0.20 for high quality studies).

Publication Bias

The funnel plot of the effect sizes of the studies was slightly skewed to the left (Fig. 3). However, the more formal Egger's test showed no evidence for publication bias ($P = 0.30$). No studies were imputed in the Trim and Fill procedure when the random effects model was used, while 3 studies were imputed when the fixed effects model was used, resulting in a mean effect size of -0.31 (95% CI -0.50 to -0.12).

Cumulative Meta-analysis

We performed a cumulative meta-analysis of the 46 trials of pain and PUFA supplementation by year of publication (data not shown). A statistically significant effect was first observed after publication of the trial by Geusens et al in 1994 (53) (SMD -0.35, 95% CI -0.68 to -0.02) and at the same point when we considered pain from rheumatoid arthritis only. Subsequent trials have increased the precision of the effect but no meaningful changes were observed in the magnitude.

Discussion

The results of our meta-analysis of intervention studies support the hypothesis that PUFA supplementation improves chronic pain moderately. The effect is

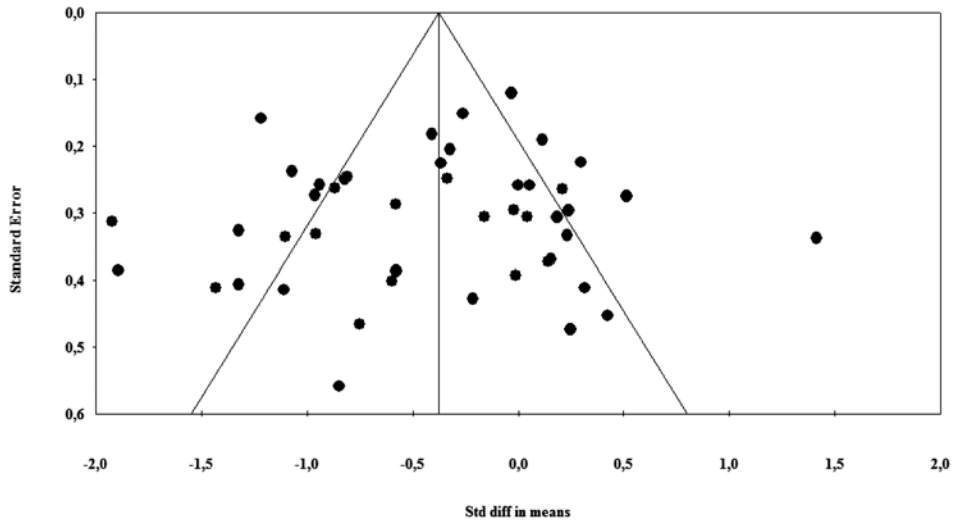

Fig. 3. *Funnel plot of standard error by standard mean difference.*

important for dysmenorrhoea and the group of "other chronic pain syndromes," less intense for rheumatoid arthritis, and absent for mastalgia. Apparently, this effect is mainly due to Ω-3, as other types of supplementation failed to exert any effect. A larger effect was observed for low doses and short periods of treatment. The small number of observational studies available did not shed any light on a potential preventive effect of fatty acids on chronic pain and only one study (cross-sectional) out of 5 was supportive of an association.

Publication bias could have affected the results of our study. This may have occurred if trials with non-significant or negative results would have remained unpublished. However, both funnel plot examination and Egger's test showed no evidence of publication bias, and the Trim-and-Fill method did not alter our results. We had to exclude 12 studies (Fig. 1) from those retrieved in our search as they were lacking essential data in order to be included in a meta-analysis, such as mean change in one or both groups (66,67), standard deviation (68) or both values (69). Our efforts to obtain these data from the authors were in vain as the large majority could not be traced. The overall conclusions of those 12 studies swayed between beneficial effects of fatty acids to harmful ones. Theoretically, inclusion of these studies could then have modified our results. Nonetheless, this possibility is highly improbable in view of the results of the "Fail-safe N" procedure that we performed subsequently. The Fail-safe N procedure computes the number of studies with null results necessary to reverse the observed association in a meta-analysis and render it statistically non-significant (70). In our case this number was 982. This number is too large for our results to be due to publication bias.

A set of meta-analyses with a limited number of studies, relating fatty acids supplementation to specific painful diseases, was published in the last decade. A meta-analysis of 9 studies, published in 2004, did not find any effect of Ω-3 supplementation on patients assessed pain in rheumatoid arthritis (71). Another meta-analysis of 14 studies (12), published in 2007, focused on inflammatory pain and Ω-3 supplementation and found a small to moderate improvement in pain-related outcomes. Finally, in 2012, Lee et al (72) published a meta-analysis of 10 studies restricted to high dose (> 2.7 g/day) and long period (> 3 months) Ω-3 supplementation in rheumatoid arthritis. The authors did not find any effect of this supplementation scheme. The outcome used in these meta-analyses was generally a proxy of pain, such as inflammatory responses, physician evaluations, or non-steroidal anti-inflammatory drugs (NSAIDs) consumption. In our meta-analysis, we included only those studies that provided direct pain measurements, such as VAS or composite scores. The larger number of studies, the inclusion of Ω-6 supplementation trials, and different dosages, intervention

periods, and diseases can explain the differences and discrepancies between the results of those previous meta-analyses and our study.

The relation between PUFA and inflammation (and its concomitant pain) is well established. Eicosanoids such as prostaglandins (PG), thromboxanes, and leukotrienes (LTs) are important mediators and regulators of inflammation. They are synthesized through the cyclooxygenase (COX) and lypoxygenase (LOX) pathways from arachidonic acid (ARA), an Ω-6 PUFA obtained from dietary sources and synthesis from linoleic and γ-linoleic acid (5). Inhibition of the COX pathway is the main action of NSAIDs. The Ω-3 PUFAs EPA and DHA inhibit the ARA pathway, leading to decreased production of eicosanoids and other inflammatory cytokines such as TNF-α, IL 1, and IL 6. Additionally, this inhibition gives rise to the synthesis of a series of leukotrienes with low inflammatory potential which may even act as antagonists of the ARA-derived mediators (6,7). Furthermore, experts consider that DHA and EPA may give rise to powerful anti-inflammatory molecules, the resolvins E1 and D1, which have analgesic effects (73).

Ω-6 PUFAs, in contrast, were generally considered to be involved in the molecular mechanism that induces pain, as prostaglandins, inflammatory eicosanoids, and cytokines derive from them. Nevertheless, some studies reported that Ω-6 PUFAs are associated with the suppression of pain and may then both inhibit and induce pain transmission (7).

Our study has some limitations. We found high heterogeneity in almost all analyses. Some degree of heterogeneity was expected, as our meta-analysis includes trials of pain outcomes from different diseases, with different supplementation patterns, doses, duration, baseline characteristics, etc. The subgroup analyses did not succeed in eliminating this heterogeneity except for the case of mastalgia, which included a small group of trials with similar characteristics.

Another limitation is the fact that, in some studies, olive oil was used as placebo. Olive oil was shown to have anti-inflammatory properties, due to the fact that oleic acid competes with arachidonic acid (74). This could have reduced the effect observed in our meta-analysis. This potential dilution effect is confirmed when we stratified our analysis by placebo type. The 38 studies that did not use olive oil in the control group showed a more intense effect of PUFA (SMD -0.43, 95% CI -0.63 to -0.23) than the 8 studies that used olive oil (SMD -0.24, -0.68 to 0.20). Our results would be biased towards the null value, which means that the effect of PUFA intake observed in our meta-analysis is underestimated.

The results of our meta-analysis show that high doses and long periods of supplementation are less effective in mitigating pain than low doses and short periods, especially in the Ω-3 group. In their meta-analysis Goldberg and Katz (12) found that long treatment periods were effective only for physician-assessed pain, and not for other outcomes, but former reviews of clinical trials, ex vivo studies, and mice models about inflammatory diseases and Ω-3 supplementation did show a dose and time-dependent relation, and ended up recommending high doses of fatty acids for long periods (5,6). The explanation of our finding and thus, the reason for this apparent lack of consistency could be threefold: 1) previous meta-analyses measure outcomes that are different from ours. While our meta-analysis assessed pain as a symptom perceived by patients, those meta-analyses measured outcomes that could be considered as proxies of pain: morning stiffness, number of painful or tender joints, and NSAID consumption, among others. Recent research showed that Ω-3 PUFA could relieve pain not only via immune regulation and inflammation suppression, but also via pain signalling inhibition and release of β-endorphins and resolvins (7). It is then plausible that the dose-response pattern varies, depending on whether subjective pain is used as an outcome, as in our meta-analysis, or another less subjective inflammation-related outcome. 2) In longer studies, patients referred decreasing their intake of NSAIDS (72). This could result in less pain relief. 3) Some placebo effect cannot be ruled out for shorter studies. A recent study suggested that the magnitude of the placebo effect could be larger in short studies than in long ones (75).

Conclusion

The large number of studies included, the magnitude of the associations found, the consistency of the results through different settings, and the existence of a mechanism that gives biologic plausibility to the relationship, provide evidence that PUFA supplementation may be moderately effective against pain, especially that associated with rheumatoid arthritis and dysmenorrhea. Existing studies on the potential preventive effect of PUFA intake are too scarce to lead to any conclusion. Future research should include careful evaluation of the dose-response relationship in clinical trials and implementation of cohort studies to assess preventive effects of PUFAs.

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