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## Insight in the role of lipids and other systemic factors in hand and knee osteoarthritis: lessons from clinical studies

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# Part 1

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**Lipids, inflammation and other metabolic factors in osteoarthritis**



# 2

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## **Fatty acids and osteoarthritis: different types, different effects**

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## Abstract

While the association between obesity and osteoarthritis used to be solely regarded as a result of increased mechanical loading, systemic factors also likely play a role in the pathophysiology of osteoarthritis. Nutrient excess leading to obesity may result in lipotoxicity, which might be involved in the development of osteoarthritis. The different fatty acid types have distinct effects on inflammation. This review focusses on the currently available studies, summarizing the effects of the different fatty acid types on osteoarthritis and involved joint tissues.

In animal studies omega-3 polyunsaturated fatty acids reduced the expression of inflammatory markers, cartilage degradation and oxidative stress in chondrocytes. In contrast, these markers were increased upon omega-6 polyunsaturated fatty acid and saturated fatty acid stimulation. Additionally, a decrease in pain and dysfunction was observed upon omega-3 supplementation in cats and dogs.

In line, most human *in vitro* studies show pro-apoptotic and pro-inflammatory actions of saturated fatty acids. While all polyunsaturated fatty acids reduced markers of oxidative stress, omega-3 polyunsaturated fatty acids additionally decreased prostaglandin production. Human intervention studies with omega-3 polyunsaturated fatty acid supplementation may indicate a beneficial effect on pain and function and might be associated with less structural damage. In contrast, an adverse effect of saturated fatty acids on osteoarthritis has been observed. Monounsaturated fatty acids have been infrequently studied and findings are inconclusive.

Existing studies indicate a promising effect of especially omega-3 polyunsaturated fatty acids on osteoarthritis signs and symptoms. However, more human intervention studies are warranted to draw robust conclusions.

## Introduction

### *Obesity and osteoarthritis, a twofold effect*

In middle-aged adults, musculoskeletal disorders are among the leading causes of disability. Osteoarthritis (OA) is a prevalent disorder, affecting 237 million people globally. The prevalence and burden of OA has greatly increased in the past decade<sup>1</sup>, and it is expected to increase even further with ageing of the population and increasing prevalence of obesity<sup>2</sup>. OA may develop in any joint, but occurs most commonly in spine, hands, knees and hips. Multiple tissues are involved in the osteoarthritic process, which is characterized by cartilage degradation, synovial inflammation and (subchondral) bone remodelling<sup>2</sup>. OA is a heterogeneous disorder that is starting to be recognized as a cluster of diseases that can be subdivided into different phenotypes, such as post-traumatic, ageing-related and metabolic OA<sup>2-4</sup>.

In the last decades a profound change in lifestyle and diet has occurred. The Western diet and the increasingly sedentary lifestyle have led to an increase in overweight and obese individuals. The association of obesity with OA has been recognized many years ago<sup>5</sup>, and has been supported by a great amount of evidence since then<sup>6-10</sup>. For long it was thought that obesity mainly resulted in an increased mechanical loading, leading to mechanical stress, increased wear-and-tear, and subsequently to cartilage degradation and OA<sup>11</sup>. However, a paradigm shift has occurred more recently, as obesity is also associated with OA in non-weight-bearing joints like the hands, in which systemic processes are likely more involved<sup>7,8,12</sup>. Therefore, systemic effects of obesity have emerged as possible players in OA. Among these, metabolic syndrome has been most intensively studied and conflicting results were reported<sup>13-18</sup>. Similarly, soluble mediators released by adipose tissue such as adipokines and fatty acids could mediate the systemic effects of obesity. Adipokines have been extensively investigated in relation to OA<sup>19</sup>. In the present review, we will focus on fatty acids and their possible involvement in OA development and progression.

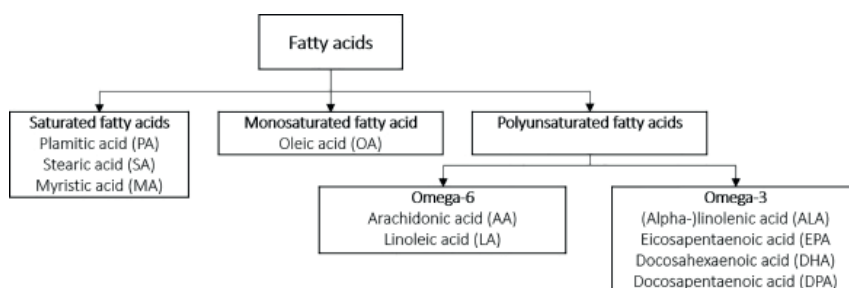
### *Lipid triggered meta-inflammation*

Excess nutrient intake may result in a lipid influx that exceeds the capacity of the adipose tissue to store lipids. Consequently, an excess of fatty acids is observed in the circulation, which is associated with the accumulation of lipids at ectopic sites such as the liver and skeletal muscle. This might lead to systemic lipotoxicity and could influence inflammatory responses. There is evidence indicating that chronic nutrient excess results in increased inflammation through cytokines such as tumour necrosis factor (TNF) and cellular receptors such as toll-like receptor (TLR)-4, and these might have an important contribution to the pathogenesis of metabolic diseases<sup>20</sup>. However, although excess fat intake is widely accepted as unhealthy, it seems that not just the quantity of fat intake plays a role, but also that the type of fat is important<sup>21</sup>.

Fatty acids can be categorized depending on their length and degree of saturation into saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The latter group can be further divided into omega-6 (n-6) and omega-3 (n-3) PUFAs (figure 1), based on the location of the last double bond relative to the terminal methyl end of the molecule. The change towards our Western diet increased our intake in SFA, decreased our intake of PUFA and increased the ratio between n-6 and n-3 PUFAs from 1-4:1,

which is deemed optimal, to a ratio of 16-20:1<sup>21</sup>. The different fatty acid types are believed to have distinct effects on inflammation. SFA and n-6 PUFAs have a more pro-inflammatory effect, while n-3 PUFAs have anti-inflammatory effects<sup>22</sup>. Therefore, it is likely that they also play different roles in metabolic OA.

The relationship between fatty acids and OA pathophysiology needs further elucidation. In this review we outline the evidence for a role of fatty acids in the development and progression of OA from studies in animals and humans. We will focus on the association of different fatty acid types with OA and their distinct effects on different joint tissues.



**Figure 1.** Fatty acid subtypes

## Methods

### *Literature search details*

We performed a PubMed literature search to obtain *in vitro*, animal and human studies describing the association of fatty acids with OA. Included search terms were; “fatty acids”, “fatty acids, omega-6”, “fatty acids, omega-3”, “fatty acids, unsaturated”, “fatty acids, monounsaturated”, “dietary fats”, “lipids”, “osteoarthritis” and “degenerative arthritis”. The search was restricted to the English language and excluded review articles.

### *Study selection*

The above described search retrieved 256 potential studies, which were screened on title and abstract to determine eligibility by one review author (ML). Fifty-five full-text papers were retrieved and assessed for eligibility to determine inclusion, resulting in a total of 29 studies relevant to the subject of this review. Additionally, three relevant articles were identified by other sources. All studies are described in table 1 and 2.

## Evidence from animal studies

### *Fatty acid treatment of chondrocytes*

A number of *in vitro* studies investigated the effect of supplementation of different fatty acids to chondrocytes of animal origin<sup>23-26</sup>. Many of these studies have investigated the effect of fatty acids on the secretion or expression of inflammatory factors, such as interleukins (IL), TNF, matrix metalloproteinase (MMPs) and/or prostaglandins. Zainal et al. pre-incubated bovine chondrocytes with the n-3 PUFAs eicosapentaenoic acid (EPA), docosahexaenoic (DHA) and alpha-linolenic acid (ALA), followed by IL-1 stimulation. They observed delayed IL-

1 $\alpha$  induced cell death in cultures pre-incubated with EPA. In addition, IL-1 induced expression of A Disintegrin And Metalloproteinase with ThromboSpondin motifs (ADAMTS)-4 and ADAMTS-5, cyclooxygenase (COX)-2, MMP-3, IL-1 $\alpha$ , IL-1 $\beta$  and TNF- $\alpha$ , were reduced upon pre-incubation with n-3 PUFAs, most potently by EPA. In contrast, the n-6 PUFA arachidonic acid had no visible effect on the expression of the investigated inflammatory markers<sup>24</sup>. Adler et al. supplemented canine chondrocytes with n-3 PUFAs, showing reduced IL-induced gene expression of inducible nitric oxide synthase (NOS). This decrease was also observed after incubation with the n-6 PUFA arachidonic acid, however, arachidonic acid additionally exerted pro-inflammatory effects, namely increased expression of ADAMTS-5 and release of prostaglandin E (PGE)<sup>25</sup>. In contrast, stimulation of mouse primary chondrocytes with the SFA stearic acid resulted in increased protein stability and transcription of hypoxia-inducible factor (HIF)1- $\alpha$ , a marker of oxidative stress<sup>23</sup>.

In summary, stimulation of chondrocytes of animal origin with n-3 PUFAs has been shown to reduce the expression of inflammatory markers, proteinases involved in cartilage degradation and markers of oxidative stress. Additionally, IL- $\alpha$  induced cell death seemed to be delayed by stimulation with the n-3 PUFA EPA. In contrast, stimulation with the n-6 PUFA arachidonic acid was shown to increase makers of cartilage degradation and inflammation and SFA increased makers of oxidative stress.

#### *Lower n-6:n-3 PUFA ratios may protect against osteoarthritis*

In a mouse model able to endogenously convert n-6 PUFAs to n-3 PUFAs (Fat-1 mice), it was shown that a reduced n-6:n-3 ratio gave a modest reduction of IL-6 and TNF- $\alpha$  production, compared to wild-type mice. However, the authors did not see any effects on idiopathic osteophyte development, synovial hyperplasia or cartilage degeneration, or on subchondral bone features<sup>27</sup>. Huang et al. utilized the same mouse model, however additionally induced OA by meniscectomy. In Fat-1 mice with surgically induced OA, they found a decrease in cartilage loss, less abrasion and less osteophytes, when compared to wild-type operated mice. They also observed a decrease in MMP-13 and ADAMTS-5 protein expression in mice with enhanced n-3 PUFA synthesis<sup>26</sup>.

Increasing n-3 PUFA levels, and thereby decreasing the n-6:n-3 PUFA ratio, might reduce expression of inflammatory makers and structural damage compared to controls in mice OA models.

#### *Supplementation of different fatty acid types yields opposite effects*

In mice with surgically induced OA, a diet high in n-3 PUFA showed less cartilage degradation, synovial hypertrophy, macrophage infiltration and heterotopic ossification, when compared to mice on diets high in SFA or n-6 PUFA. Also, n-3 PUFA fed mice showed lower bone mineral density (BMD) compared to mice on the other diets and controls<sup>28</sup>. In line, in OA prone guinea pigs, dietary n-3 PUFAs ameliorated OA associated histologic features<sup>29</sup>. In addition to their work on chondrocytes, Miao et al. also investigated the effect of a high-fat diet on plasma levels of inflammatory markers in mice. They observed increased production of IL-6, TNF- $\alpha$  and IL-1 $\beta$  compared in mice on a high-fat diet, to mice on a normal diet<sup>23</sup>. Sekar et al. investigated the relative effect of SFA chain length, and observed that a diet with longer chain SFAs, such as palmitic acid and stearic acid, induced greater cartilage degeneration, increased MMP-13 and collagenase (COL)10 expression and increased apoptosis, compared to mice fed a diet with shorter chain SFAs<sup>30</sup>.



A cross-over study with cats with radiographically diagnosed OA, showed increased owner-reported activity and less stiffness upon a fish oil diet high in n-3 PUFA, compared to cats on a corn oil diet high in n-6 PUFA. However, no wash-out period was used between the diets tested<sup>31</sup>. The duration of the effect of dietary supplemented fatty acids is not well known, therefore effects of the first diet may have interfered with the effects of the second diet. Randomised controlled, double blinded trials in dogs have shown that dogs with OA showed less lameness and discomfort<sup>32,33</sup> and less functional disability when fed a n-3 rich diet compared to dogs fed a control diet<sup>34,35</sup>. Fritsch et al. investigated the need for the painkiller carprofen in dogs with OA, upon a diet high in n-3 PUFA compared to a control diet low in n-3 PUFA. They observed that carprofen dosages could be decreased faster in dogs receiving n-3 PUFAs<sup>36</sup>. A study comparing fish oil and corn oil supplementation showed improvement of markers of oxidative stress in both groups. In contrast to the other studies they failed to show a difference between diets<sup>37</sup>.

In conclusion, various studies have investigated the effects of fatty acid enriched diets. A diet high in n-3 PUFA has been shown to reduce OA associated structural damage, compared to a diet high in SFA or n-6 PUFA. Studies also showed that n-3 supplementation resulted in owner-reported decreased dysfunction and less need for pain medication when compared to a regular control diet. In contrast, high fat diet or a diet high in SFA increased the expression of inflammatory markers, which was increased even further with SFA with longer chain length. Additionally, an increase in apoptotic chondrocytes was found upon a diet high in SFA.

**Table 1a.** Main outcomes of animal in-vitro studies

Author, year	Study population	FA	Methods	Main outcomes
Adler, 2017 <sup>25</sup>	Canine chondrocytes	n-3 PUFA n-6 PUFA	Incubation with FA, stimulated with IL-1 $\beta$	N-6 and n-3 PUFA reduced IL-induced iNOS gene expression and NO production. N-6 PUFA reduced MMP-3, increased ADAMTS-5 and PGE.
Huang, 2014 <sup>26</sup>	Mouse primary chondrocytes	n-3:n-6	Incubation with FA	N-3 PUFA decreased mTORC1 activity and promoted autophagy.
Miao, 2015 <sup>23</sup>	Mouse primary chondrocytes	SFA	Stimulation with SFA	SFA increased protein levels, stability and transcription activity of HIF1a in primary chondrocytes.
Zainal, 2009 <sup>24</sup>	Bovine chondrocytes	n-3 PUFA n-6 PUFA	Pre-incubation with FA followed by IL-1 stimulation	EPA delayed cell death. All n-3 PUFAs reduced IL-1 induced ADAMTS-4, COX2, IL-1 $\alpha$ and TNF- $\alpha$ expression. EPA and ALA reduced ADAMTS-5 expression. EPA reduced expression of MMP-3 and MMP-13. No effect on COX1 expression. N-6 PUFA showed no effect on gene expression.

ADAMTS = A Disintegrin And Metalloproteinase with ThromboSpondin motifs, ALA = alpha-linolenic acid, COL = collagenase, COX = cyclooxygenase, DHA = docosahexaenoic acid, ECM = extracellular matrix, EPA = eicosapentaenoic acid, GAG = glycosaminoglycan, HIFs = hypoxia-inducible factors, HFD = high fat diet, IL = interleukin, MA = myristic acid, MMP = matrix metalloproteinase, n-3 = omega-3, n-6 = omega-6, NOS = nitric oxide synthase, OA = osteoarthritis, n = number, PA = palmitic acid, PGE2 = prostaglandin E2, PUFA = polyunsaturated fatty acid, SA = stearic acid, SFA = saturated fatty acid, SF = synovial fluid, TLR = toll-like receptor, TNF = tumour necrosis factor, VAS = visual analogue score, VEGF = vascular endothelial growth factor, vs = versus, WT = wild-type

**Table 1b.** Main outcomes of animal intervention studies

Author, year	Study population	FA	Methods	Main outcomes
Barrouin-Melo, 2016 <sup>37</sup>	Dogs with OA, n = 77	n-3 PUFA n-6 PUFA	Fish oil vs. corn oil supplementation	Fish oil and corn oil improved markers of oxidative stress and decreased monocytes, no clear differences between the groups.
Cai, 2014 <sup>27</sup>	Fat-1 (n = 17) vs. WT mice (n = 18), idiopathic OA	n:6:n3 PUFA	n-6 PUFA enriched diet	Fat-1 expression reduced n-6:n-3 compared to WT. IL-6 and TNF- $\alpha$ levels were modestly reduced in Fat-1 mice. No differences in histologic changes or bone morphology.
Corbee, 2013 <sup>31</sup>	Cats with OA, n = 16 mean age 13y	n-3 PUFA	Fish oil vs. corn oil (0% EPA and DHA)	Cats on fish oil showed higher activity, less stiffness and more interaction compared to corn oil.
Fritsch, 2010 <sup>36</sup>	Dogs with OA, n = 131	n-3 PUFA	Fish oil vs. control low n-3 diet	Carprofen dosage decreased faster among dogs fed n-3 diet compared to control diet
Huang, 2014 <sup>26</sup>	Fat-1 vs. WT mice, n = 24/ group, surgically induced OA.	n-3:n-6	-	Decreased n-6:n-3 reduced cartilage degeneration and osteophytes, decreased MMP-13, ADAMTS-5, reduced chondrocyte number and loss of ECM.
Knott, 2011 <sup>29</sup>	OA prone guinea pigs, n = 10/ group	n-3 PUFA	N-3 enriched vs. standard diet	N-3 PUFA reduced histology scores, increased GAG and decreased denatured type II collagen and decreased active MMP-2, compared to standard diet. Subchondral bone parameters changed towards normal.
Mehler, 2016 <sup>32</sup>	Dogs with OA, n= 74, mean age 7.8y	n-3 PUFA	Triglyceride n-3 oil vs. placebo oil	No differences in total joint score and improvement VAS compared to placebo. Discomfort and lameness scales improved after 84 days.
Miao, 2015 <sup>23</sup>	Lean and obese C57BL/6 mice	-	HFD	HFD increased plasma IL-6, TNF- $\alpha$ , IL-1 $\beta$ and VEGF. No histologic OA signs. Effects partly mediated through HIF1a and TLR4.
Moreau, 2012 <sup>34</sup>	Dogs with OA, n=15 / group, mean age 6.5y	n-3 PUFA	N-3 diet vs. control	Improvement of functional disability and performance in activities of daily living in n-3 PUFA diet group, not in controls.
Roush, 2010 <sup>33</sup>	Dogs with OA, n = 38	n-3 PUFA	3,5% fish oil vs control	Dogs fed n-3 diet showed significant improvements in lameness and weight bearing after 3 months, compared to baseline.
Roush, 2010 <sup>35</sup>	Dogs with OA, n =127	n-3 PUFA n-6:n-3	N-3 rich n-6:n-3 low diet vs. control diet	N-3 enriched diet improved owner-reported ability to rise, play and walk, compared to control diet.
Sekar, 2017 <sup>30</sup>	Rats, n = 12 /group Chondrocytes	SFA	Corn starch diet vs. HFD with different SFAs	Rats fed beef tallow, SA, MA or PA diets exhibited cartilage degradation and subchondral bone changes. More cartilage degradation, MMP-13, COL10 expression and apoptosis in longer chain SFAs.
Wu, 2015 <sup>28</sup>	Mice, n = 49, surgically induced OA	SFA n-3 PUFA n-6 PUFA	HFD SFA-rich, n-6 rich or n-3 rich vs control	N-3 PUFA enriched HFD reduced surgically-induced OA and enhanced wound repair. SFA or n-6 PUFA increased OA severity, with increased cartilage damage, thicker synovium, heterotopic ossification and were associated with macrophage infiltration.

ADAMTS = A Disintegrin And Metalloproteinase with Thrombospondin motifs, ALA = alpha-linolenic acid, COL = collagenase, COX = cyclooxygenase, DHA = docosahexaenoic acid, ECM = extracellular matrix, EPA = eicosapentaenoic acid, GAG = glycosaminoglycan, HIFs = hypoxia-inducible factors, HFD = high fat diet, IL = interleukin, MA = myristic acid, MMP = matrix metalloproteinase, n-3 = omega-3, n-6 = omega-6, NOS = nitric oxide synthase, OA = osteoarthritis, n = number, PA = palmitic acid, PGE2 = prostaglandin E2, PUFA = polyunsaturated fatty acid, SA = stearic acid, SFA = saturated fatty acid, SF = synovial fluid, TLR = toll-like receptor, TNF = tumour necrosis factor, VAS = visual analogue score, VEGF = vascular endothelial growth factor, vs = versus, WT = wild-type

## Evidence from human studies

### *Beneficial effects of n-3 fatty acid supplementation on chondrocytes and synoviocytes*

Six studies investigated the effect of *in vitro* stimulation of human chondrocytes and/or synoviocytes<sup>23,38-42</sup>. Alvarez-Garcia et al. incubated chondrocytes isolated from human articular cartilage with fatty acids. They found no effect of mono-incubation of chondrocytes with fatty acids. However, when they co-incubated chondrocytes with IL-1 $\beta$ , they observed an increase in caspase 3 and 7, and decreased chondrocyte viability upon incubation with the SFA palmitate. They did not see this effect upon co-incubation with oleate, a MUFA. In addition, they observed increased IL-6, COX-2 and NOS2 mRNA levels in both normal and OA chondrocytes upon incubation with palmitate, but not oleate. Incubation of human synoviocytes showed similar results. Furthermore, they found that these effects were reduced by inhibition of caspase or TLR-4 signalling<sup>38</sup>. In contrast, Bastiaansen-Jenniskens et al. observed a decrease in MMP-1 expression and glycosaminoglycan release upon treatment of human chondrocytes not only with oleate, but also with the SFA palmitate when co-stimulated with TNF- $\alpha$ . Additionally, oleate decreased prostaglandin-endoperoxide synthase (PTGS2). They did not observe effects on the investigated inflammatory and destructive markers when chondrocytes were stimulated only with SFA, MUFA or n-6 PUFA<sup>39</sup>. Frommer et al. investigated the effect of SFA and PUFA stimulation on IL-6 secretion, both in human chondrocytes and synovial fibroblasts. In human chondrocytes the strongest effect was observed upon stimulation with the SFA palmitic acid, while unsaturated fatty acids, especially PUFAs, had much weaker effects on IL-6 secretion. In synovial fibroblasts they found a dose-dependent increase in IL-6 secretion both upon stimulation with SFA and PUFA. The SFA stearic acid has also been shown to enhance expression of IL-6, TNF- $\alpha$ , IL-1 $\beta$  in chondrocytes, compared to controls. This study supported the finding from Alvarez-Garcia et al.<sup>38</sup> that the SFA induced inflammation is partially mediated by TLR-4<sup>40</sup>. Shen et al. treated human osteoarthritic chondrocytes for 6 days with different n-6 PUFAs, (conjugated) linoleic acid and arachidonic acid, and the n-3 PUFA EPA. They observed a lower PGE2 production in chondrocytes treated with n-3 PUFA, however not significantly. Stimulation of NO production with IL-1 and LPS was significantly reduced by all investigated PUFA treatments, compared to the control group<sup>41</sup>.

In summary, in line with studies with chondrocytes from animal origin, most human *in vitro* studies show pro-apoptotic and pro-inflammatory actions of SFA. These actions seem to be at least partially mediated by TLR-4 signalling. Although MUFA have been studied less often, most studies show no effect of cell treatment with MUFA. N-3 PUFAs have been shown to reduce prostaglandin production by human chondrocytes, while all PUFAs reduced makers of oxidative stress. Although results are comparable to those observed in animal studies, n-3 PUFAs have been studied less often in human *in vitro* studies.

**Table 2a.** Main outcomes of human in-vitro studies

Author, year	Study population	FA	Methods	Main outcomes
Alvarez-Garcia, 2014 <sup>38</sup>	Human chondrocytes and synoviocytes	SFA MUFA	Incubation with FA alone, or co-incubation with IL- $\beta$	Co-incubation of chondrocyte with SFA and IL- $\beta$ increased caspase 3 and 7. SFA but not MUFA increased IL6, cox2 and nos2 mRNA and IL-6 secretion. In human synoviocytes SFA, not MUFA increased IL-6 and COX2 expression. Effects reduced by inhibition of caspase or TLR-4.
Bastiaansen-Jenniskens, 2013 <sup>39</sup>	Chondrocytes isolated from TKR material. Knee OA, n= 3, mean age 72y, mean BMI 31, 67% women	SFA MUFA n-6 PUFA	Stimulation with FA alone, or co-incubation with TNF $\alpha$	Without TNF- $\alpha$ : no effect of FA on MMP-1, PTGS2 and PGE2, but decrease of GAG by oleic acid and PA, not LA. With TNF- $\alpha$ : decrease of MMP-1 gene expression by oleic acid and PA, no effect of LA. Oleic acid decreased PTGS2, no effect of LA and PA. LA increased PGE2, no effect of oleic acid and PA. Oleic acid and PA lowered GAG release, no effect of LA.
Frommer, 2013 <sup>40</sup>	Synovial fibroblasts from RA, OA, PsA and controls. Primary chondrocytes	SFA PUFA	Stimulation with FFA	Both SFA and PUFA increased IL-6 secretion dose-dependently. Findings seem to be mediated by TLR4.
Humphries, 2012 <sup>44</sup>	Cancellous bone matrix from OA patients	SFA MUFA n-3 PUFA n-6 PUFA	Gas chromatography of FFA profile	Subchondral bone from OA subjects had higher n-6 and n-3 PUFA than controls, no difference in n-6:n-3 ratio, or MUFA.
Lippiello, 1991 <sup>43</sup>	Femoral heads from THR in OA patients, n=21	SFA n-6 PUFA MUFA	Gas chromatography of FFA levels	85% of total lipid content was comprised of palmitic, linoleic and oleic acid. Increasing FFA levels with increasing OA severity, no differences between FFA type.
Miao, 2015 <sup>23</sup>	Human chondrocytes from TKR material, n=10, mean age 65y	SFA	Stimulation with SFA	SFA treated chondrocytes showed increased IL-6, TNF- $\alpha$ , IL-1 $\beta$ and VEGF mRNA levels, compared to controls.
Shen, 2004 <sup>41</sup>	Human osteoarthritic chondrocytes	CLA, combined with n-6 or n-3 PUFA	6 days pre-incubation with FA	Compared to control, supplementation of CLA or LA alone and LA/EPA resulted in lower PGE2 production. Incubation with CLA/EPA resulted in lowest PGE2 production. LA/AA, LA/EPA, and CLA treatments decreased IL- $\beta$ induced NO, LA and CLA/EPA treatments increased NO production.
Yu, 2015 <sup>42</sup>	Human chondrocytes	n-6:n-3	Treatment with FA	Low n-6:n-3 decreased MMP-13 mRNA and protein levels in human chondrocytes. No effect on chondrocyte proliferation

AA = arachidonic acid, AdA = adrenic acid, ALA = alpha-linolenic acid, BMI = body mass index, BMD = bone mineral density, BML = bone marrow lesion, CFA = cetylated fatty acid, CLA = conjugated linoleic acid, COX = cyclooxygenase, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, FFA = free fatty acid, GAG = glycosaminoglycan, IL = interleukin, JSW = joint space width, LA = linolenic acid, MMP = matrix metalloproteinase, MUFA = monounsaturated fatty acid, n-3 = omega-3, n-6 = omega-6, NOS = nitric oxide synthase, OA = osteoarthritis, n = number, PA = palmitic acid, PGE2 = prostaglandin E2, PLSDA = partial least-squares-discrimination analysis, PUFA = polyunsaturated fatty acid, RA = rheumatoid arthritis, RCT = randomized controlled trial, ROM = range of motion, SFA = saturated fatty acid, SF = synovial fluid, TLR = toll-like receptor, TNF = tumour necrosis factor, UPLC-MC = ultra-performance liquid chromatography mass spectrometry, VEGF = vascular endothelial growth factor, vs = versus

**Table 2b.** Main outcomes of human epidemiological and intervention studies

Author, year	Study population	FA	Methods	Main outcomes
Baker, 2012 <sup>52</sup>	Knee OA, n = 472, mean age 60y, mean BMI 30, 50% women	n-3 PUFA n-6 PUFA	Cross-sectional	Multivariable logistic regression controlling for age, sex and BMI, showed positive relation between plasma levels of n-3 PUFAs with the amount of patellofemoral cartilage, but not tibiofemoral cartilage or synovitis. Positive association between n-6 PUFA and synovitis.
Castro-Perez, 2010 <sup>46</sup>	Hip and knee OA, n = 59, 100% women	-	Lipidomics UPLC-MS	Multivariable PLSDA suggested altered lipid metabolism associated with OA.
Chen, 2016 <sup>53</sup>	Knee OA, n = 202, mean age 61y, mean BMI 29, 49% women	n-3 PUFA	RCT, double blind. High vs low dose n-3 fish oil	No differences in BMD after 2 years between high and low dose groups after adjusting for baseline BMD.
Gruenewald, 2009 <sup>48</sup>	Hip and knee OA, n = 178, mean age 62y, mean BMI 29, 63% women	n-3 PUFA	RCT, double blind. Glucosamine sulphate + n-3 PUFA vs glucosamine sulphate alone	Greater reductions in morning stiffness and pain (WOMAC) upon combination therapy vs. glucosamine sulphate alone.
Hesslink, 2002 <sup>49</sup>	Knee OA, n = 66, mean age 58y, mean BMI 28, 33% women	CFA	RCT, double blind. CFA vs placebo (vegetable oil)	CFA increased knee flexion, but not extension, compared to placebo. Patient-reported pain reduction and improvement of disability after 68 days compared to placebo.
Hill, 2016 <sup>50</sup>	Knee OA, n = 202, mean age 61y, mean BMI 29, 49% women	n-3 PUFA	RCT, double blind. High dose vs low dose fish oil	Greatest reduction in WOMAC pain and disability after 2 years in low-dose group. No difference in cartilage volume, BML score, NSAID use and quality of life.
Jónasdóttir, 2017 <sup>45</sup>	Knee OA, n = 24, Mean age 68y, mean BMI 29, 16% women	-	Targeted lipidomics LC-MS/MS	N-6 PUFAs AA, AdA, LA and the n-3 PUFAs EPA, DPA and ALA were detected in SF of OA patients.
Kraemer, 2004 <sup>51</sup>	Knee OA, n = 40, mean age 64y, mean BMI 31, 85% women	CFA	RCT, double blind. Topical cream with CFA or placebo	CFA topical cream improved knee ROM, ability to ascend/descend stairs, ability to rise from sitting, walk and sit down, and unilateral balance.
Lu, 2017 <sup>54</sup>	Radiographic knee OA, n = 2092, mean age 62y, 59% women	SFA MUFA PUFA	Longitudinal	Self-reported total fat and SFA intake was positively associated with JSW loss at 48 months. Higher intakes of MUFA, PUFA and higher PUFA to SFA ratio was associated with reduced JSW loss.
Zhang, 2015 <sup>47</sup>	Knee OA, n = 40, healthy controls. Mean age 58y, mean BMI 28, 50% women	-	Metabolomics UPLC-MS	OA-specific serum metabolic profile enabled discriminating patients with knee OA from age- and gender-matched controls and different severities of OA.

AA = arachidonic acid, AdA = adrenic acid, ALA = alpha-linolenic acid, BMI = body mass index, BMD = bone mineral density, BML = bone marrow lesion, CFA = cetylated fatty acid, CLA = conjugated linoleic acid, COX = cyclooxygenase, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, FFA = free fatty acid, GAG = glycosaminoglycan, IL = interleukin, JSW = joint space width, LA = linolenic acid, MMP = matrix metalloproteinase, MUFA = monounsaturated fatty acid, n-3 = omega-3, n-6 = omega-6, NOS = nitric oxide synthase, OA = osteoarthritis, n = number, PA = palmitic acid, PGE2 = prostaglandin E2, PLSDA = partial least-squares-discrimination analysis, PUFA = polyunsaturated fatty acid, RA = rheumatoid arthritis, RCT = randomized controlled trial, ROM = range of motion, SFA = saturated fatty acid, SF = synovial fluid, TLR = toll-like receptor, TNF = tumour necrosis factor, UPLC-MC = ultra-performance liquid chromatography mass spectrometry, VEGF = vascular endothelial growth factor, vs = versus

### *Fatty acid levels in OA patients*

In the beginning of the nineties of the last century, Lippiello et al. investigated the distribution profile of individual fatty acids in articular cartilage of femoral heads obtained from total hip replacement surgery. They showed that total fatty acid levels, and increased arachidonic acid levels in particular, were positively associated with the severity of osteoarthritic lesions<sup>43</sup>. In line, higher percentages of total n-6 and n-3 PUFAs were observed in subchondral bone of OA patients, when compared to patients with fractured neck of femur, and control subjects. No differences in the ratio between n-6 and n-3 PUFAs, nor in the percentage of MUFAs was observed in the subchondral bone of the different patient groups<sup>44</sup>.

Characterization of lipid profiles of individual patients and identification of biomarkers may aid early OA diagnosis. Jónasdóttir et al. investigated FA in synovial fluid of knee OA patients. They detected seven different PUFAs; the n-6 PUFAs adrenic acid and linoleic acid (LA) and the n-3 PUFAs EPA, DHA and docosapentaenoic acid (DPA)<sup>45</sup>. Castro-Perez et al. demonstrated using ultra performance liquid chromatography mass spectrometry (UPLC-MS) that they were able to differentiate between patients without structural hip or knee OA and patient with early and moderate OA. Multivariable partial least-squares-discrimination analysis (PLSDA) suggested an altered lipid metabolism in OA patients, compared to patients with knee complaints without OA abnormalities on radiography<sup>46</sup>. Using a similar approach, Zhang et al. discriminated knee OA patients from healthy age- and gender-matched controls and were able to stratify patients on OA severity. They observed involvement of multiple metabolic pathways, including fatty acid and lipid metabolism<sup>47</sup>.

Investigation of the fatty acids present in OA tissues showed that in more severe osteoarthritic lesions the total fatty acid levels and in particular the n-6 PUFA levels were increased. Current lipidomic research seems promising for the development of biomarkers. However, how these findings relate to dietary fatty acid intake is difficult to establish. Unfortunately, research in this field is still rather limited. However, these studies may be valuable to identify potential biomarkers to help diagnose OA in an early disease-stage, therefore additional research is warranted.

### *Effect on pain and function*

The efficacy of fatty acids on OA symptoms has been investigated in four human intervention studies, which investigated almost exclusively patients with knee OA<sup>48-51</sup>. Supplementation of cetylated fatty acids (CFA), compared to placebo consisting of vegetable oil, resulted in a significant increase in knee flexion, and a modest improvement in patient-reported outcomes. However, the consistency of the placebo vegetable oil was not described<sup>49</sup>, therefore possible effects of the placebo treatment cannot be ruled out. CFA has also been investigated as a topical treatment. Application of cream with CFA showed improvement in range of motion of knee OA patients after 30 days, which was not observed in patients receiving placebo cream<sup>51</sup>. Gruenwald et al. investigated in a randomized, double blind controlled trial the effects of adding n-3 PUFAs to glucosamine sulphate therapy in hip and knee OA patients. They found a higher number of patients with reported WOMAC pain reduction after 26 weeks, compared to mono-therapy with glucosamine sulphate<sup>48</sup>. The beneficial effect of n-3 PUFAs is supported by Hill et al., who compared high-dose versus low-dose fish oil supplementation in knee OA patients. They observed improvement in WOMAC pain and function in both groups. However, they found a greater improvement in the low-dose fish oil group after 2 years. They did not observe any differences in NSAID use or quality of life. Of note, the low-dose fish oil supplement was a blend of fish oil and sunola oil. The effect of sunola oil on the measured

outcomes is not clear<sup>50</sup>.

In conclusion, human intervention studies with n-3 PUFA supplementation indicate a possible beneficial effect of n-3 PUFAs on patient reported outcomes such as pain and function. However, studies struggled to include a proper control group. Placebo treatment consisted of a different kind of (vegetable) oil, of which the consistency was not always described. It is hard to predict the treatment effect of these placebo treatments. Therefore, results should be interpreted with caution.

**Table 3.** The effect of fatty acids on different joint tissues

Fatty acid	Cartilage	Synovium	Subchondral bone
Saturated fatty acid	Increased plasma TNF- $\alpha$ , IL-1 $\beta$ , VEGF <sup>23</sup> in mice, IL6 secretion in mice and humans <sup>23, 38, 40</sup> and il6, cox2, nos2 mRNA and caspase 3 and 7 expression in humans <sup>38</sup> , Increase in cartilage degradation <sup>26,30</sup> more severe in longer chain SFA <sup>30</sup> in mice and rats. Associated with JSW loss in humans <sup>54</sup> .	Synovial hypertrophy and macrophage infiltration in mice <sup>28</sup> . Increased IL-6 and COX2 expression in human synoviocytes <sup>38</sup> .	Increased heterotopic ossification in mice <sup>28</sup> .
Monounsaturated fatty acid	Increased IL6 expression <sup>40</sup> , no effect on il6, cox2 and nos2 mRNA <sup>38</sup> in human chondrocytes. Associated with reduced JSW loss in humans <sup>54</sup> .	Not reported	Not reported
Polyunsaturated fatty acid	Increased expression of ADAMTS-5, iNOS and PGE in canine chondrocytes <sup>25</sup> . Reduced NO production in animal and human chondrocytes <sup>25,41</sup> . Increased cartilage degradation in mice <sup>28</sup> and with reduced JSW loss <sup>54</sup> in humans.	Synovial hypertrophy and macrophage infiltration in mice <sup>28</sup> . In humans a positive association with MRI assessed synovitis was found <sup>52</sup>	Not reported
Omega-6	Reduced NO and iNOS production in canine chondrocytes <sup>25</sup> . Reduced expression of ADAMTS-4 <sup>24</sup> and ADAMTS-5 <sup>24,26</sup> , COX-2, MMP-3, IL-1 and TNF- $\alpha$ <sup>24</sup> in chondrocytes of animal origin. Delayed cell death <sup>24</sup> . Reduced PGE2, NO <sup>41</sup> and MMP-13 production <sup>42</sup> in human chondrocytes. Reduced cartilage degradation <sup>26</sup> in mice. In humans a reduced amount of patellofemoral, but not tibiofemoral cartilage <sup>52</sup> and reduced JSW loss <sup>54</sup> , but not all showed effect on cartilage volume <sup>50</sup> .	In humans no effect observed <sup>52</sup> .	In mice less osteophytes were observed upon lowering n-6:n-3 ratio <sup>26</sup> , which was not seen by others <sup>27</sup> . In humans no effect on BMLs <sup>50</sup> or BMD was observed <sup>53</sup> .
Omega-3			

ADAMTS = A Disintegrin And Metalloproteinase with Thrombospondin motifs, BMD = bone mineral density, BML = bone marrow lesion, COX = cyclooxygenase, IL = interleukin, JSW = joint space width, MMP = matrix metalloproteinase, MRI = magnetic resonance imaging, NOS = nitric oxide synthase, PGE = prostaglandin E, SFA = saturated fatty acid, TNF = tumour necrosis factor, VEGF = vascular endothelial growth factor

### *Fatty acids and structural OA abnormalities*

Additionally, four human studies have investigated structural abnormalities upon fatty acid supplementation (table 3)<sup>50,52-54</sup>. In multivariable models adjusting for several potential confounding factors, including BMI, weight changes and baseline disease severity, Lu et al. observed effects of reported dietary intake of fatty acids on joint space width (JSW) loss over 4 years' time. Total fatty acid intake and SFA intake was positively associated with JSW loss. Opposite effects were found for MUFA and PUFA. Higher unsaturated fatty acid intake was associated with reduced JSW loss<sup>54</sup>. Baker et al. cross-sectionally investigated the association of plasma n-6 and n-3 PUFAs with semi-quantitatively scored synovial thickening and cartilage amount in knee OA patients in the MOST study. In multivariable models adjusting for age, sex and BMI, they observed an positive relation between n-3 PUFAs and amount of

patellofemoral cartilage, but not tibiofemoral cartilage or synovial thickness. In addition, they found a positive association between the n-6 PUFA arachidonic acid and synovial thickness<sup>52</sup>. However, in a randomized controlled trial by Hill et al., no differences in cartilage volume or BML scores assessed by MRI were found between patients receiving high-dose or low-dose fish oil supplementation<sup>50</sup>. Another randomized, double blind clinical trial investigated the effect of high-dose and low-dose n-3 supplementation on BMD measured by DEXA. They found no differences in BMD between the groups after 2 years<sup>53</sup>.

The effect of fatty acids on structural abnormalities in OA has been investigated rarely. To be able to draw more robust conclusions additional research is warranted.

## Discussion and conclusion

Animal *in vitro* and intervention studies suggest unfavourable effects of SFA, with increased production of pro-inflammatory and pro-apoptotic markers. Conversely, n-3 PUFAs have been shown to decrease markers of inflammation and cartilage degradation. Multiple animal intervention studies have shown a beneficial effect of a diet high in n-3 PUFAs or with a low n-6:n-3 PUFA ratio, with a decrease in cartilage degradation and less osteophyte formation. However, the study designs and research methods employed often preclude an unequivocal interpretation of the results. Studies utilized owner-reported outcomes, which are difficult to interpret regarding reliability. In addition, due to unknown effects of other supplementation diets, appropriateness of control treatment was questionable in most designs. Moreover, negative results have been reported very infrequent, which may indicate publication bias.

Studies in humans have been rather limited so far. An adverse effect of total fatty acids and SFAs has been observed on structural abnormalities. In addition, n-3 PUFAs may be associated with less structural damage and improvement in pain and function in knee and hip OA patients. Due to a variety in the regular diet to which the fatty acids are supplemented, fatty acid intake is less well controlled in human studies compared to animal studies. However, this will also be the case in real-life, and is therefore well generalizable to daily-practise. Unfortunately, current research in OA patients is limited to patients with knee and hip OA. It would be of great interest to see if similar results can be obtained in patients with hand OA, since in particular in hand OA patients systemic factors, such as fatty acid levels, are very likely involved in the development and progression of the disease.

In conclusion, fatty acids appear to have effects on both symptoms and structural abnormalities associated with osteoarthritis. The different fatty acid types exert distinct effects. N-3 PUFAs seem to reduce inflammatory markers and cartilage degradation. In contrast, opposed effects were observed of SFAs and n-6 PUFAs. However, to be able to draw indisputable conclusions, additional research with high quality methods, in a broader array of osteoarthritis patients will be needed.

## Research agenda

- More high-quality human intervention studies investigating the effect of fatty acid supplementation on OA are needed to draw firm conclusions.
- The involvement of fatty acids needs to be investigated in different OA phenotypes, especially in hand OA.
- The effect of MUFAs on OA requires further investigation.



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