Osteoarthritis and Cartilage

OARSI Osteoarthritis RESEARCH SOCIETY INTERNATIONAL

Review

Nutraceuticals: do they represent a new era in the management of osteoarthritis? — a narrative review from the lessons taken with five products

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SUMMARY

Objectives: The aim of this first global systematic review on selected nutraceuticals was to synthesize and evaluate scientific relevant data available in the literature. Evidences that can support health, physiological or functional benefit on osteoarthritis (OA) were gathered and the level of evidence relative to each of these ingredients was highlighted.

Methodology: Relevant scientific data (positive or not) regarding OA were searched for five groups of compounds (avocado/soybean unsaponifiables (ASU), n-3 polyunsaturated fatty acids, collagen hydrosylates (CHs), vitamin D, polyphenols) within preclinical (*in vitro* and *in vivo*), epidemiological, and clinical studies. The following criteria were evaluated to assess the methodology quality of each study: (1) study question; (2) study population; (3) primary endpoint; (4) study design (randomization, control, blinding, duration of follow up); (5) data analysis and interpretation. A scientific consensus was determined for all studied nutraceuticals to evaluate their efficacy in OA.

Results: The studied compounds demonstrated different potencies in preclinical studies. Most of them have demonstrated anti-catabolic and anti-inflammatory effects by various inhibitory activities on different mediators. Vitamin D showed a pro-catabolic effect *in vitro* and the polyphenol, Genistein, had only anti-inflammatory potency. The evaluation of the clinical data showed that ASU was the only one of the studied ingredients to present a good evidence of efficacy, but the efficient formulation was considered as a drug in some countries. Pycnogenol showed moderate evidence of efficacy, and vitamin D and collagen hydrolysate demonstrated a suggestive evidence of efficacy, whereas curcumin, epigallocatechin-3-gallate (EGCG) and resveratrol had only preclinical evidence of efficacy due to the lack of clinical data. The literature gathered for n-3 PUFA, nobiletin and genistein was insufficient to conclude for their efficacy in OA.

Conclusion: Additional data are needed for most of the studied nutraceuticals. Studies of good quality are needed to draw solid conclusions regarding their efficacy but nutraceuticals could represent good alternates for OA management. Their use should be driven by any recommendations.

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Introduction

Osteoarthritis (OA) is the most prevalent joint disease. It causes pain and disability in a large proportion of the population worldwide. It is considered as the most consequential rheumatic condition in terms of social-economic impacts. The incidence of the disease increases with age. The disease evolves over decades to end by the loss of joint function. In addition, aging patients present various co-morbid conditions that add to the complexity of the treatment of OA patients.

To date, there is no cure for OA. The only available treatments aim at reducing symptoms, as pain and inflammation, maintain joint mobility and limit the loss of function. The main goals that the ideal OA treatment has to achieve are symptom-modifying effect, reducing pain and inflammation, and structure-modifying effect, sparing joint structure and preventing joint degradation in order to maintain articular function.

Several guidelines, as the European League Against Rheumatism (EULAR), the American College of Rheumatology (ACR) or the

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Osteoarthritis Research Society International (OARSI) recommendations for the management of knee OA[1–4] have been published for OA management. They all recommend both non-pharmacological and pharmacological approaches. The non-pharmacological interventions include education and self-management, regular telephone contact, referral to a physical therapist, aerobic, muscle strengthening and water-based exercises, weight reduction, walking aids, knee braces, footwear and insoles, thermal modalities, transcutaneous electrical nerve stimulation and acupuncture. The pharmacological treatments consist of acetaminophen, cyclo-oxygenase-2 (COX-2) non-selective and selective oral non-steroidal anti-inflammatory drugs (NSAIDs), topical NSAIDs and capsaicin, intra-articular injections of corticosteroids and hyaluronates, glucosamine and/or chondroitin sulphate, and avocado/soybean unsaponifiables (ASU) for symptom relief; glucosamine sulphate, chondroitin sulphate and diacerein for possible structure-modifying effects and the use of opioid analgesics for the treatment of refractory pain[1,5]. Most of the pharmacological treatments available to relieve OA symptoms present serious adverse events, as the risk of gastro-intestinal or cardiovascular adverse events with NSAIDs.

Moreover, this disease implies treatment or drug intake for decades, increasing the risk of serious adverse events and the incidence of co-morbidity factors.

Many efforts have been developed to find a cure to OA that can satisfy all the above-mentioned goals. The perfect drug would be not only able to relieve inflammation and pain but also to slow down, stop or even better prevent disease progression. This would result in the maintenance of joint function, sparing joint structures involved in OA, meaning cartilage, synovial membrane and subchondral bone.

From the molecular point of view, OA joints are the site of inflammation and catabolism. Many key mediators have been identified in cartilage for both pathways. Inflammation is linked to interleukin (IL)-1β, COX-2 expression and prostaglandin E_2 (PGE_2) and nitric oxide (NO) production. Catabolism results from an imbalance with anabolism. The synthesis of catabolic enzymes as different matrix metalloproteinases (MMP-1, 3 or -13) or the disintegrin and metalloprotease with thrombospondine motifs (ADAMTS)-4 and -5 (also known as aggrecanases) is increased resulting in the degradation of the main cartilage matrix components (proteoglycan (PG) and type II collagen). In parallel, the synthesis of the matrix components is decreased. Synovial inflammation is directly linked to cartilage degradation. In addition, subchondral bone is the site of strong remodeling processes resulting in bone sclerosis. All these factors produce the loss of the articular integrity and the loss of joint function.

Indeed, there is a strong necessity for prevention of OA. The first step passes by healthy lifestyle, weight loss and nutrition, with specific nutrients that could help to achieve this goal. Nutraceuticals are good candidates to help patient preventing OA or managing their disease using them as treatment adjuvant.

Nutraceutical comes from the combination of the words nutrition and pharmaceutical. It corresponds to food or food product that provide health and medical benefits, including prevention and treatment[6,7]. By definition and regulatory laws they are devoid of adverse effects.

Nutraceuticals are good candidates for long-term prevention of chronic disease, such as OA. Many compounds have already been studied and a review by *Ameye and Chee*[6] has gathered all the scientific data available at that time. There are several emerging alternatives. It is more and more recognized that nutraceuticals could help to maintain bone and joint health. However this is paramount to give a critical point of view to judge the quality of the studies. Nutraceuticals are under minimal and vague regulation. Dietary supplements do not have to be approved by the US Food and Drug Administration (FDA). Nutraceuticals are monitored as dietary supplement within the US and the definition for functional foods varies depending on countries. In Europe the situation is different with an ongoing regulatory reform tightening the existing regulatory framework. Indeed, European Food Safety Authority (EFSA) adopted Regulation 1924/2006 on the use of nutrition and health claims for food in December 2006. This regulation harmonised rules across the European Union (EU) for the use of health or nutritional claims on foodstuffs, which are based on nutrient profiles. One of its key objectives is to ensure that all claims made on food labels in the EU are "clear and substantiated by scientific evidence". EFSA is responsible for verifying the scientific substantiation of the submitted claims, some of which are currently in use, some of which are proposed by applicants. This information serves as a basis for the European Commission and Member States, which will decide whether to authorize each individual product claim. EFSA started to release opinions in October 2009 on health claims submitted under Article 13 of the regulation, covering so-called generic health claims.

The aim of this first global systematic review on selected nutraceuticals was to synthesize and evaluate scientific relevant data available in the literature. Evidences that can support health, physiological or functional benefit on OA were gathered and the level of evidence relative to each of these ingredients was highlighted.

Methodology

Relevant scientific data (positive or not) regarding OA were searched for five groups of compounds (Table I) within *in vitro* (Table III), *in vivo* (Table IV), epidemiological (Table V), and clinical studies (Table VI). The selection of compounds discussed in this paper is arbitrary and was based mainly on the following criteria: amount of emerging science, safety of use, regulatory constrains (Novel Food), natural presence in food. The objective was in the end to identify ingredients that could support joint health, but also that could be authorized in food and would be relevant to be delivered through a food matrix.

We have voluntary eliminated glucosamine sulphate, glucosamine-HCl, and chondroitin sulphate because these natural compounds are considered as drugs in some countries, and that they have been the main topic of numerous systematic review and meta-analysis[5,8–13]. The search was performed according to the following criteria: (1) only scientific data with a direct link to OA were selected; (2) only orally administered treatments were selected for *in vivo* studies and clinical trials (CTs); (3) only publications in English were considered (4) only scientific data allowing to evaluate the effect of the compound alone were considered. Articles describing the results of a study previously published were excluded. The search was performed in Pubmed/Medline database between January 1990 and 2010.

This search was performed using the combination of terms related to OA (arthrosis or osteoarthr* OR gonarthro* OR coxarthro* OR "joint pain" OR "joint comfort" or chondro* or fibroblast* OR
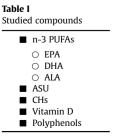
Table I
Studied compounds

■ n-3 PUFAs
○ EPA
○ DHA
○ ALA
■ ASU
■ CHs
■ Vitamin D
■ Polyphenols

Table II
Results of Pubmed data search performance

Compound	Total number of publications retrieved	Total number of publications selected	Nb of *in vitro* studies	Nb of *in vivo* studies	Nb of observational CTs	Nb of interventional CTs
n-3 PUFAs	1508	9	4	2	1	2
ASU	59	17	9	4	0	4
CHs	51	7	2	1	0	4
Vitamin D	2249	9	3	1	5	0
Pine bark extract	13	4	0	0	0	4
Prodelphinidins	1	1	1	0	0	0
Nobiletin	10	3	3	0	0	0
Genistein	571	3	2	1	0	0
EGCG	81	7	7	0	0	0
Resveratrol	146	6	5	1	0	0
Curcumin	231	12	12	0	0	0
Ventol	2	1	1	0	0	0
Quercetin	210	1	1	0	0	0

synov* OR subchond* OR cartilage OR collagen) and each studied compound name (or abbreviation).

The methodological quality of each CT supporting functional ingredient efficacy was determined according to an assessment model adapted from EFSA and FDA recommendations (EFSA, 2007; FDA, 2003), AFSSA ("*Agence Française de Sécurité Sanitaire des Aliments*") guidelines (AFSSA, 2007) and other relevant references[6,14,15] (ANAES "*Agence Nationale d'Accréditation et d'Evaluation en Santé*", 2000). The quality is scored according to a set of 14 criteria. One point is marked for each criterion presented in the description of the CT. The points are then summed and the final score allow classifying the CT quality in four different categories: a score below six represents a poor methodological quality, from 7 to 9 represents a medium methodological quality, from 10 to 11 represents a good methodological quality and finally a score from 12 to 14 represents a very good methodological quality.

A scientific consensus was reached between the two evaluators (YH and CL) by considering different points: the total number of CTs (showing or not a beneficial effect), the quality of these CTs, the number of epidemiological studies showing or not a relationship, the heterogeneity in the body of evidence, the presence of preclinical basis and the presence of ongoing CTs. The studies were then classified as good evidence of efficacy, moderate evidence of efficacy, limited evidence of efficacy but suggestive, preclinical evidence of efficacy, lack of evidence of efficacy, some evidence of inefficacy. Scientific consensus for each ingredient is summarized in Table VII.

Furthermore, a search of ongoing CTs (Table VIII) was carried out on the *clinicaltrials.gov* database using the term "osteoarthritis" and each studied compound's name, in order to complete existing published data and give an overview of current research interest on the selected ingredients.

Results

Bibliographic search results

The results of the search performed on Pubmed/Medline database are described in Table II.

N-3 polyunsaturated fatty acids (n-3 PUFAs)

n-3 PUFAs (linolenic acid and eicosapentenoic acid (EPA)) are essential fatty acids. These compounds are candidate for the reduction of inflammation as they can substitute arachidonic acid (main precursor of prostaglandins) in the synthetic pathway of inflammatory mediators. The reduction of inflammation can also have an impact on the catabolic pathways and by that way on disease progression. They have been widely studied in cardiovascular and inflammatory diseases as rheumatoid arthritis[16,17]. These studies demonstrated the beneficial effects of a higher n-3 intake. It is important to note that Western diet is richer in n-6 PUFAs (linoleic acid and arachidonic acid) rather than in n-3 PUFAs[6].

In vitro and preclinical data

n-3 PUFAs have been extensively studied in various cell types, but only few studies have assessed their anti-inflammatory or anti-OA effects in joint cell models. Three *in vitro* studies have been identified using bovine chondrocytes or human and bovine cartilage explants. These studies used n-3 PUFAs alpha-linolenic acid (ALA), EPA and docohexanoic acid (DHA)[18–20]. These studies demonstrated the potency of n-3 PUFAs at reducing inflammatory mediators (IL-1α, COX-2, 5-lipoxygenase (LOX) and its activator FLAP) and also catabolic factors (MMPs or ADAMTS). Furthermore, recent published data have also shown that n-3 PUFAs reduced IL-1β-induced ADAMTS-4, -5, MMP-3, -13 and COX-2 mRNA in bovine chondrocytes culture[21].

Similarly, only one animal study on n-3 PUFAs and OA has been identified in the literature[22]. This study investigated the effect of n-3 PUFAs on rats with a marginally deficient essential fatty acid state. n-3 PUFA produced a 70% maximum decrease in the linoleic and arachidonic acid content of articular cartilage. n-3 PUFA also produced a 30−40% decrease in the cartilage hexosamine content and a 32% inhibition of PG synthesis. This is important to keep in mind that this study demonstrated that a too low n-6/n-3 ratio can be negative, as a diet with very low n-6 PUFAs intake induced surface irregularities and PG depletion in cartilage of rats[6,22].

Finally, the only one *in vivo* study testing n-3 PUFAs in client-owned OA dogs was just published[23]. The results are in favor of the beneficial effect of n-3 PUFAs on OA dogs.

Epidemiological data

One observational study was identified. This study investigated using Magnetic resonance Imaging (MRI), the association of different fatty acids consumption with cartilage structure and bone marrow lesions (which have been shown to be associated with knee pain and predictive of cartilage loss in knee OA)[24]. The main observation of this study showed that high intakes of monounsaturated, total and n-6 PUFAs are associated with increased risks of bone marrow lesions. The results made no association between n-3 PUFAs and either bone marrow lesions or cartilage volume or defects.

Clinical data

The clinical data on n-3 PUFAs are limited. Two studies that investigated the effect of natural extract or oil with high content of n-3 PUFAs on OA symptoms were identified. One study of medium quality (score: 7)[25] showed that a daily intake of 10 ml of cod liver

Table III
Summary of the *in vitro* effects of the studied products on OA

Reference	Product	Dose and incubation duration (ID)	*In vitro* model	Results
n-3 PUFAs				
Curtis et al. 2000[18]	EPA, DHA or ALA	10−100 µg/ml ID: 8 h	Bovine chondrocytes	Dose-dependent reduction of IL-1α induced-aggrecanase expression and activity Reduction of IL-1α, TNF-α and COX-2 (with ALA) expression
Curtis et al. 2002[19]	ALA or EPA	10−100 µg/ml ID: 24 h	Human OA cartilage explants	Reduction of endogenous and IL-1-induced release of PG metabolites in a dose-dependant manner by n-3 PUFA but not by n-6 PUFA Suppression of the endogenous proteolytic activity of aggrecanase and collagenase by n-3 PUFAs Suppression of the mRNA expression of ADAMTS, MMP-3 and MMP-13 by n-3 PUFA No effect of n-3 PUFA on TIMP-1, -2 and -3
Curtis et al. 2002[20]	EPA or ALA	10−300 µg/ml 24 h pre-treatment with EPA or ALA and 4 days ncubation with IL-1β	Normal bovine or osteoarthritic human cartilage explants stimulated with IL-1β	Reduction of IL-1-induced inflammation and catabolism (reduction of GAG release, and of aggrecanase activity, loss of COX-2 and 5-LOX expression) by n-3 PUFA No effect of n-3 PUFA on normal tissue homeostasis
Zainal et al. 2009[21]	EPA, DHA or ALA	2.5−30 µg/ml 8 h pre-treatment and 4 days incubation with IL-1β	Normal bovine articular chondrocytes stimulated with IL-1β	Demonstration of the chondrocyte ability to incorporate exogenous PUFAs Reduction of IL-1b-induced production of cartilage degradating enzyme (agrecanases, MMPs) and inflammatory cytokines Inhibition of COX-2 by n-3 PUFAs but not COX-1
ASU				
Mauviel et al. 1989[33]	ASU	0.1−10 µg/ml ID: 24 h and 8−14 days	Synoviocytes Rabbit articular chondrocytes	No impact of ASU alone on collagen synthesis in synoviocytes and chondrocytes after 24 hInhibition of IL-1β-induced collagen synthesis decrease in synoviocytes after 24 hStimulation of collagen synthesis on articular chondrocytes after 8−14 days
Mauviel et al. 1991[34]	ASU Piascledine®	10 µg/ml ID: 48 h	Rabbit articular chondrocytes Human rheumatoid synovial cells	Slight increase of collagen production in both cell types Partial inhibition of IL-1β-induced release of collagen in synovial cells and total suppression in chondrocytes
Henrotin et al. 1998[35]	ASU mixed in three ratios 1:2 (A1S2) 2:1 (A2S1) 1:1 (A2S2)	10 µg/ml ID: 72 h	Human chondrocytes	Reduction of the stromelysin (MMP-3), IL-6, IL-8 and PGE₂ spontaneous productionDecrease of the collagenase activity in unstimulated and stimulated chondrocytes by A1S2Partial inhibition of IL-1 effects
Boumediene et al. 1999[39]	ASU	10−25 µg/ml	Bovine articular chondrocytes	Stimulation of the expression of TGFβ1, TGFβ2, plasminogen activating inhibitor-1 (PAI-1)
Henrotin et al. 2003[36]	ASU (A1S2)	0.625−40 µg/ml ID: 12 days	Human OA chondrocytes stimulated or not with IL-1β	Stimulation of aggrecan production and restoration of aggrecan production after IL-1β stimulation Decrease of basal and IL-1β-stimulated MMP-3 production Weak inhibition of IL-1β −induced TIMP reduction Inhibition of basal production of MIP-1β, IL-6, IL-8, NO and PGE₂ Stimulation of TIMP-1 production
Henrotin et al. 2006 (abstract)[40]	ASU	10 µg/ml ID: 72 h	Human OA chondrocytes co-cultured or not with osteoblasts (obtained from sclerotic (SC) or non-sclerotic (NSC) zones of OA subchondral plate	Prevention of the inhibitory effects of SC osteoblasts on matrix components by pre-treatment of SC osteoblasts with ASU Increase of type II collagen mRNA level in co-culture with ASU-pretreated SC osteoblasts No modification of MMP, TIMP-1, TGF-b1, TGF-b3 or iNOS expression and COX-2 mRNA levels in chondrocytes when co-cultured with ASU-pretreated SC osteoblasts
Au et al. 2007[38]	ASU	25 µg/ml ID: 72 h	Chondrocytes THP-1 monocytes/macrophages	Reduction of TNF-α, IL-1β, COX-2 and iNOS expression in LPS-stimulated chondrocytes to non-activated control levels Reduction of PGE₂ production and COX-2 and iNOS expression Reduction of TNF-α in LPS-stimulated monocyte/macrophage

Lippiello et al. 2008[32]	Sterols extracted from three ASU preparations	1−10 µg/ml (sterols) ID: 48 h	Bovine chondrocytes	Upregulation of non-collagenous protein and collagen synthesis as well as of labelled sulphate uptake Inhibition of IL-1-induced MMP-3 activity, PGE$_2$ synthesis and sulphate release
Gabay et al. 2008[37]	ASU	10 µg/ml	Mouse or human chondrocytes stimulated with IL-1b Cartilage submitted to a compressive mechanical stress (MS)	Decrease of MMP-3 and -13 expression and PGE$_2$ synthesis Inhibition of the degradation of IκBα and suppression of NF-κB translocation Inhibition of Erk 1/2 but no effect on the other IL-1β-induced MAPK

CH

Oesser and Seifert, 2003[50]	CH	0.5 mg/ml ID: 48 h	Bovine chondrocytes	Dose-dependant increase of type II collagen secretion No increase in type II collagen secretion by native collagens and collagen-free hydrosylate of wheat proteins (used as control) No effect on the expression of proteases
Schunck et al. 2006[51]	CH Glucosamine	Not provided (NP)	Porcine articular chondrocytes Human femoral head chondrocytes	Increase of PG synthesis, aggrecan expression and type II collagen biosynthesis with CH No effect of glucosamine on extracellular matrix macromolecules (glucosamine sulphate and hydrochloride)

Vitamin D

Tetlow and Woolley, 1999[66]	1,25-dihydroxy-vitamine D3	10^{-8} M ID: 48 h	Rheumatoid synovial fibroblasts (RSF) stimulated or not with IL-1β Human articular chondrocytes stimulated or not with IL-1β	No effect on spontaneous MMP and PGE$_2$ production by RSF Reduction of IL-1β-induced MMP and PGE$_2$ production (up to 50%) by RSF Slight reduction of spontaneous MMP-1 and -3 production by chondrocytes No effect on IL-1β-induced MMP and PGE$_2$ production and stimulation of IL-1β-induced MMP-3 production by chondrocytes
Cantatore et al. 2004[60]	1,25-dihydroxy-vitamine D3	10^{-8} M ID: 48 h	Osteoblasts from OA subchondral bone samples	Increased stimulation of osteoclacin production by maximally damaged Osteoblasts compared to minimally damaged ones
Tetlow and Woolley, 2001[65]	1,25-dihydroxy-vitamine D3	10^{-8} M ID: 48 h	Human articular chondrocytes stimulated with TNF-α or phorbol myristate acetate (PMA)	No effect on MMP-1, -9 and PGE$_2$ production Upregulation of MMP-3 with or without stimulation with TNF-α or PMA

Polyphenols

Ishiwa et al. 2000[78]	Nobiletin	NP ID: NP	Rabbit synovial fibroblasts Rabbit articular chondrocytes	Suppression of IL-1-induced MMP-9 mRNA expression and production Reduction of IL-1-induced PGE$_2$ production No modification of the synthesis of total protein
Imada et al. 2008[77]	Nobiletin	16−24 µM ID: 24 h	Normal human synovial fibroblasts	Suppression of IL-1β-induced ADAMTS-4 and -5 mRNA expression
Lin et al. 2003[79]	Nobiletin	6−64 µM ID: 24 h	Normal human synovial fibroblasts	Suppression of IL-1β-induced production of PGE$_2$ in a dose-dependant manner Selective downregulation of COX-2 but not COX-1 mRNA expression Downregulation of IL-1β-induced gene expression and production of pro-MMP-1 and pro-MMP-3 Increased production of the endogenous MMP inhibitor, TIMP-1

Williamson et al. 2006 (abstract)[86]	Resveratrol and/ or Curcumin	2.5 µM ID: 5 days	LPS-stimulated canine cartilage explants	Decrease of GAG release by curcumin alone and in combination with resveratrol
Lev-Ari et al. 2006[92]	Curcumin	0−20 µM ID: 72 h	OA synovial adherent cells	Increased inhibitory effect of celecoxib on COX-2 activity Stimulation of the growth-inhibitory and anti-apoptotic effects of celecoxib
Schulze-Tanzil et al. 2004[82]	Curcumin	50 µM ID: 12−48 h	Human chondrocytes stimulated with IL-1β	Prevention of IL-1β-induced MMP-3 upregulation Inhibition of IL-1β-induced-type II collagen synthesis suppression Prevention of NF-κB translocation
Shakibaei et al. 2005[84]	Curcumin	50 µM ID: 5−30 min	Human articular chondrocytes	Anti-apoptotic and anti-catabolic effects on IL-1β-stimulated chondrocytes

Table III (continued)

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Reference	Product	Dose and incubation duration (ID)	*In vitro* model	Results
Liacini et al. 2003[81]	Curcumin	10−15 μM ID: 24 h	Human OA chondrocytes	Inhibition of TNF-α-induced MMP-13 gene expression
Shakibaei et al. 2007[83]	Curcumin	50 μM ID: 72 h	Human articular chondrocytes stimulated with IL-1β	Suppression of NF-κB mediated IL-1b or TNF-a catabolic signalling pathways resulting in COX-2 and MMP-9 downregulation and type II collagen upregulation
Toegel et al. 2008[85]	Curcumin	5−50 μM ID: 24−48 h	Immortalized human chondrocytes (C-28/I2) stimulated with IL-1β	No effect on aggrecan and type I and II collagen gene expression, proliferation and morphology at low concentrations Cell damages at high concentrations (reduction of cell viability) Increase of type II collagen, MMP-3 and ADAMTS-4 expression and decrease of type I collagen expression with high concentrations
Clutterbuck et al. 2008 (abstract)[89]	Curcumin	25−100 μM ID: 5 days	Equine cartilage explants stimulated with IL-1β	Reduction of IL-1β-induced GAG release (50−100 μM) Decrease of PGE_2 release (25−100 μM)
Clutterbuck et al. 2009[87]	Curcumin	0.1−100 μM ID: 5 days	Equine cartilage explants stimulated with IL-1β	Suppression of IL-1β-induced GAG release
Mathy et al. 2007 (abstract)[88]	Curcumin	1−30 μM ID: 24 h	Primary bovine chondrocytes stimulated with IL-1β	No effect on cell viability Dose-dependant inhibition of IL-1β-induced COX-2, iNOS, IL-6 and IL-8 gene expression, PGE_2 and NO production
Chowdhury et al. 2008[91]	Curcumin	0.01−1000 ng/ml ID: 48 h	Bovine chondrocytes cultured in agarose	Inhibition of IL-1β-induced NO and PGE_2 release Inhibition of $^{35}SO_4$ incorporation
Mathy-Hartert et al. 2009[90]	Curcumin	5−20 μM ID: 12 days	Human articular chondrocytes in alginate beads and human cartilage explants stimulated with IL-1β	No effect on cell viability Dose-dependent reduction of the synthesis of inflammatory mediators (NO, PGE_2, IL-6, IL-8) and catabolic factor (MMP-3)
Claassen et al. 2008[94]	Genistein	10^{-11}−10^{-4} M ID: 24 h (for incubation or preincubation)	Foetal bovine articular chondrocytes	No effect on GAG release within the physiological range of concentrations Decrease of GAG release with high doses (10^{-5}−10^{-4} M) No effect on sulphate incorporation by chondrocytes Preincubation with 10^{-9}−10^{-5} M enhanced the stimulatory effect of insulin on sulphate incorporation by chondrocytes
Hooshmand et al. 2007[95]	Genistein	50−100 mM ID: 1 h preincubation+24 h	Human chondrocytes stimulated by LPS	Reduction of pro-inflammatory molecules (COX-2 and NO) No effect of YKL-40 (marker of cartilage metabolism) or IL-1β levels
Singh et al. 2002[98]	EGCG	1−100 μM ID: 30 min preincubation+24 h	Human OA chondrocytes stimulated with IL-1β	Inhibition of IL-1β-induced NO production by interfering with NF-κB activation
Ahmed et al. 2002[97]	EGCG	100−200 μM ID: 2 h preincubation+24 h or 24 h without preincubation	Human OA chondrocytes stimulated with IL-1β	Dose-dependant inhibition of NO and PGE_2, iNOS and COX-2 expression
Singh et al. 2003[99]	EGCG	5−200 μM ID: 24−48 h	Human OA chondrocytes stimulated with IL-1β	Suppression of IL-1β-induced upregulation of catabolic mediators dependant on the activation of c-jun N-terminal kinase (JNK) activation
Rasheed et al. 2009[102]	EGCG	25-200 μM ID: 1−2 h pre-treatment with EGCG+4 days treatment with AGE	Human OA chondrocytes stimulated with AGE	Inhibition of AGE-induced expression of TNF-α and MMP-13 Attenuation of the AGE-induced MAP kinase signalling pathways Inhibition of NF-κB activation
Ahmed et al. 2004[103]	EGCG	1−200 μM ID: 72 h	Human cartilage explants and chondrocytes	Inhibition of IL-1β-induced GAG release from human cartilage explants Dose-dependant inhibition of MMP-1 and MMP-13 IL-1β-induced mRNA and protein expression in human chondrocytes Dose-dependant inhibition of transcription activity of NF-κB and AP-1
Tokuda et al. 2008 (abstract)[100]	EGCG	NP	Osteoblast-like MC3T3-E1 cells	Inhibition of the fibroblast growth factor (FGF)-2-stimulated synthesis of IL-6 at least in part through the suppression of p44/p42 and the p38 Map kinase pathways
Huang et al. 2009[101]	EGCG	10−50 μM ID: 12 h	Human synovial fibroblasts stimulated with IL-1β	Inhibition of IL-1β-induced COX-2 expression and synthesis Inhibition of IL-1β-induced PGE_2 and IL-8 secretion

Williamson et al. 2006 (abstract)[86]	Resveratrol and/or Curcumin	2.5 μM ID: 5 days	Canine cartilage explants stimulated with LPS	Decrease of GAG release by resveratrol alone Inhibition of the degradative effects of LPS and decrease of GAG release by co-treatment with resveratrol and curcumin
Dave et al. 2008[105]	Resveratrol	1−10 μM ID: 1 h pre-treatment	Human OA chondrocytes and cartilage explants stimulated by IL-1β	Inhibition of IL-1β-induced and spontaneous of PGE_2 and LTB_4 production, and suppression of COX-2 expression Spontaneous stimulation of PG and inhibition of pro-MMP-13 Prevention of OA cartilage degradation from IL-1β-induced effects Prevention of chondrocytes apoptosis
Csaki et al. 2008[106]	Resveratrol	1−200 μM ID: 1−24 h	Human articular chondrocytes stimulated by IL-1β	Chondroprotective effect through suppression of IL-β-, reactive oxygen species (ROS)- and tumor suppressor protein p53-production
Shakibaei et al. 2008[107]	Resveratrol	100 μM ID: 4 h (pre-treatment + 1−32 h (in combination with IL-1β)	Human articular chondrocytes stimulated by IL-1β	Inhibition of IL-1β-induced vascular endothelial growth factor (VEGF), MMP-3, MMP-9 and COX-2 Suppression of apoptosis and inflammatory signalling by acting on NF-κB
Lei et al. 2008[108]	Resveratrol	100 μM ID: 24 h	Rat bone marrow mesenchymal stem cells derived chondrocytes stimulated by IL-1β and cultures on chitosan-gelatine scaffolds (CGS)	Inhibition of IL-1β-induced downregulation of type II collagen and aggrecan and increased of MMP-13 expression by reducing the translocation of NF-κB Spontaneous decrease of type II collagen but no effect on aggrecan and MMP-13 expression
Garbacki et al. 2002[111]	Prodelphidins isolated from Ribes nigrum Gallocathechin trimer (CG-CG-CG), dimer (CG-CG) or monomer (CG-ECG)	1−100 μg/ml ID: 5 min−12 days	Human chondrocytes	Stimulation of PG and type II collagen production by all tested prodelphidins Decreased of PGE_2 synthesis by all prodelphidins Inhibition of COX-2 but not COX-1 by all prodelphidins
Sato et al. 1997 (abstract)[112]	Quercetin	NP	Human synovial cells	Suppression of TNF-α mediated stimulation of IL-8 and MCP-1 expression, at least in part by inhibiting NF-κB activation
Kang et al. 2004 (abstract)[113]	Ventol	NP	Cartilage explants	Inhibition of IL-1β-induced PG degradation

IkB: inhibitor of NF-κB.

Table IV
Summary of the *in vivo* effects of the studied products on OA

Reference	Product	Dose and ID	*In vivo* model	Results
n-3 PUFAs				
Lippiello et al., 1990 (abstract)[22]	n-3 PUFA (menhaden fish oil)	Diet with 10% menhaden fish oil ID: NP	Male Sprague−Dawley with a "marginally deficient" essential fatty acid state	70% maximum decrease in articular cartilage content of the linoleic and arachidonic acid in the fish oil treated group 30−40% decrease in cartilage hexosamine content and 32% inhibition of PG synthesis
Roush et al., 2010[23]	n-3 PUFA	Diet with 31-fold increase of the total omega-3 fatty acids ID: 24 weeks	Client-owned dogs with OA $N = 127$	Significant improvement of dog conditions
ASU				
Cake et al., 2000[28]	ASU	900 mg/weekday vs placebo ID: 6 months	Ovine model of knee OA (bilateral lateral meniscectomy) $N = 32$	Reduction of subchondral bone sclerosis and increase of PG content and of articular knee joint thickness
Altinel et al., 2007[42]	ASU	300 mg every day or every 3 days ID: 3 months	Sheepdogs Control: normal diet $N = 24$	Increase of both TGF-β1 and TGF-β2 levels in knee joint fluid
Kawcak et al., 2007 [41]	ASU (A1:S2)	NP ID: 70 days	Experimentally induced OA in horses $N = 16$	No effect on pain and lameness Reduction of the severity of cartilage erosion and synovial haemorrhage Increase of articular cartilage GAG synthesis
Boileau et al., 2009[29]	ASU	10 mg/kg/day ID: 8 weeks	Experimental knee dog model	Decrease of the size of the macroscopic lesions (tibial plateaus) compared to control Decrease in the severity of cartilage lesions (tibial plateaus and femoral condyles) Decrease in the scores of all histological parameters (structural changes, cellularity, Safranin-O staining and pannus invasion on the femoral condyles) No difference on the tibial plateaus for Safranin-O and pannus invasion Reduction of iNOS production in cartilage Reduction of the total histological changes and cellular infiltration in synovium
CH				
Oesser et al., 2008 (abstract)[52]	CH (Fortigel, Gelita AG)	0.15 mg/g of body weight daily ID: 4 months	Male STR/ort (model of naturally occurring OA) Control: albumin	Decrease of cartilage tissue degeneration in knee joints Decrease of the incidence of severe joint degradation and in the determinate grade of OA in comparison to the untreated control
Vitamin D				
Jefferies et al., 2002[67]	25-hydroxyvitamin D3 supplement	0.1 mg/kg/day ID: 21 weeks	Pigs $N = 200$ Control: commercial diet	No effect on the incidence or severity of OA lesions, articular PG or collagen contents
Polyphenols				
Ham et al., 2004[96]	Genistein Soy phytoestrogen	Equivalent of 129 mg/day for women ID: 3 years	Monkey model of naturally occurring OA after ovariectomy	No effect on insulin-like growth factor binding protein (IGFBP)-2 and IGFBP-3, total protein, PG or collagen levels in cartilage tissue
Elmali et al., 2005[110]	Resveratrol	10 μmol/kg Intra-articular	Rabbit model of OA by transaction of the anterior cruciate ligament	Protection against cartilage degradation (histological evaluation)

Table V
Summary of the epidemiological data for the studied products on OA

Reference	Product	Population	Design	Results
n-3 PUFAs				
Wang et al., 2008[24]	Different types of fatty acid	Australian healthy middle-aged subjects without clinical knee OA Mean y: 58 N = 297	Cohort Y: 10	Higher intakes of monounsaturated fatty acids, total and n-6 PUFAs associated with increased risk of bone marrow lesions No association of n-3 PUFA intake and bone marrow lesions No association of fatty acid intake with cartilage volume of defects
Vitamin D				
McAlindon et al., 1996[58]	Vitamin dietary intake and serum levels of 25-hydroxyvitamin D	English patients Mean y: 70.3 N = 556	Cohort Y: 9–10	Modest correlation between serum vitamin D and vitamin D intake Increased risk of knee OA progression (global score including joint space narrowing, osteophytosis and sclerosis) for low levels of vitamin D and vitamin D intake Association between low vitamin D serum levels and knee loss of cartilage as assessed by joint space and osteophyte growth (disease progression) No association between vitamin D intake and serum level of vitamin D and knee OA incidence
Lane et al., 1999[68]	Serum levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D	American elderly white women Y ≥ 65 N = 237	Cohort Y: 8	Association between low serum levels of 25-hydroxyvitamin D and radiographic changes for hip OA characterized by joint space narrowing but not osteophyte No association between serum 1,25-dihydroxyvitamin D and incident changes of radiographic hip OA
Felson et al., 2007[69]	Serum levels of 25-hydroxyvitamin D	English adults Mean y: 53.1 N = 715	Cohort Y: 9	No association between vitamin D levels and structural joint degradation (disease incidence) defined as joint space loss on radiography
Felson et al., 2007[69]	Serum level of 25-hydroxyvitamin D	American adults with knee OA Mean y: 66.2 N = 388	Cohort Y: 9	No association between vitamin D levels and structural disease worsening (disease progression) defined as joint space loss on radiography and as cartilage loss on MRI
Breijawi et al., 2009[64]	Serum levels of 25-hydroxyvitamin D	Patients undergoing total hip or knee replacement Mean y: 69–70 N = 117	Cross-section	High prevalence of low vitamin D status in patients with knee OA
Bergink et al., 2009[63]	Serum levels of 25-hydroxyvitamin D	Knee OA patients N = 1248	Cohort Y: 6.5 (mean follow up time)	Low dietary vitamin D intake the risk of progression of knee OA Vitamin D influences the incidence and progression of knee OA more particularly in low BMD patients

oil (equivalent to 786 mg EPA) by OA patients as an adjunct to NSAID medication was not effective to improve pain and ability. The use of olive oil as placebo control may have introduced a bias in the result of this study. On the contrary, the second study of low methodological quality (score: 4)[26] showed that the consumption of an extract of New-Zealand green-lipped mussel rich in n-3 PUFAs improved OA symptoms, including pain and joint function in Korean OA patients.

ASU

ASU are derived from unsaponifiable residues of avocado and soybean oils, generally mixed in the ratio one-third–two-thirds respectively[27–30]. The large majority of the *in vitro* and *in vivo* data have been obtained with ASU found in Piascledine®300 (Laboratoires Expanscience, France). The ASU contained in Piascledine®300 are extracted according to a patented process giving them a particular formulation[30]. This formulation is considered as a drug in France and some of the observed beneficial effects seem to be related to this particular formulation. In other

countries, ASU mixtures are delivered as over-the-counter products. Therefore, extrapolation of the data obtained with Piascledine®300 to other ASU mixtures must be done with an extreme caution. The main components of ASU are the phytosterols β-sitosterol, campesterol and stigmasterol[31].

In vitro and preclinical data

Over the last 20 years, ASU have raised a great research interest. *In vitro* data are abundant. Most of *in vitro* studies were performed with normal or OA chondrocytes stimulated or not with IL-1β, the key pro-inflammatory cytokine in OA physiopathology. These studies demonstrated that ASU contained in Piascledine®300 exerted positive effects on human chondrocytes by stimulating the synthesis of aggrecan and extracellular matrix component as type II collagen[31–33] and by reducing the production of catabolic (MMP-3) and pro-inflammatory (IL-8 and IL-6) mediators[34]. These anabolic and anti-catabolic effects were also observed in human OA chondrocytes[35]. What is more, this ASU mixture was able to counteract IL-1β-induced deleterious effects on cartilage in normal and OA chondrocytes[31–36]. It reversed IL-1β-induced collagen

Table VI
Summary of the clinical data of the studied products on OA

Reference	Product	Dose and intervention duration (ID)	Population	Design	Results	Score (1–14)
n-3 PUFAs						
Stammers et al., 1992[25]	Cod liver oil (EPA)	10 ml (786 mg EPA)/dayID: 24 weeks	English middle-ages and old patients with OA Y: 49–87 N = 86	Double-blind, placebo-controlled trial	No effect on pain and ability compared to olive oil (Cod liver oil was used as an adjunct to NSAIDs)	7
Cho et al., 2003[26]	Lyprinol® extract from New-Zealand green-lipped mussel rich in n-3 PUFAs (EDA, DHA, DPA)	four capsules/day ID: 8 weeks	Korean patients with hip or knee OA Y: 40–75 N = 60	Multicenter open trial	Improvement of OA signs and symptoms (pain VAS, joint function LFI)	4
ASU						
Lequesne et al., 2002[43]	Piascledine®	300 mg (capsule) ID: 2 years	French patients with regular pain due to primary hip OA Y: 50–80 N = 108	Prospective, multicenter, randomized, parallel group, double-blind, placebo-controlled trial	No structural effect (joint space width) (primary outcome) Reduction of the progression of joint space loss in the subgroup with advanced space narrowing (post-hoc analysis) No difference for clinical parameters (secondary outcomes) between ASU and placebo groups (LFI, global pain on VAS, NSAID use and patient's global assessment) over the first year follow up	11.5
Appelboom et al., 2001[44]	Piascledine®	300 or 600 mg ID: 3 months	Belgian patients with primary knee OA under analgesics and/or NSAIDs Y:45–80	Prospective multicenter, double-blind, randomized, parallel group, placebo-controlled trial	Decrease of NSAID and analgesic intake (primary outcome), compared to placebo from the first month No difference between 300 mg and 600 mg Decrease of LFI (improvement noticed from the second month) and pain by VAS	11
Maheu et al., 1998[45]	Piascledine®	300 mg (capsule) ID: 6 months + 2 month post)treatment follow up	French patients with symptomatic primary hip or knee OA Y: 45–75 N = 164	Prospective, randomized, double-blind, placebo-controlled multicenter trial	Decrease of LFI scores (primary outcome) after 6 months, compared to baseline and placebo groups Reduction of pain by VAS, overall functional disability and patient's overall assessment efficacy More important improvement in hip OA patients No difference in NSAID consumption except for the period ranging from 6 to 8 months Beneficial effects measured by LFI, pain by VAS and functional disability started after 2 months (delayed action) Prolonged effect of ASU, persisting 2 months after treatment discontinuation	14
Blotman et al., 1997 (abstract)[46]	Piascledine®	300 mg ID: 3 months	French patients with symptomatic knee or hip OA requiring NSAID therapy Mean y: 62.9 N = 163	Prospective, randomized, double-blind, placebo-controlled, parallel-group trial	Reduction of NSAID consumption (primary outcome) Improvement of LFI compared to placebo No difference in pain score between ASU and placebo	
CH						
Clark et al., 2008[54]	CH (liquid formulation)	10 g/day ID: 24 weeks	American physically active healthy adults without degenerative joint disease but with joint pain Mean y: 20.1 N = 97	Prospective, randomized-placebo-controlled, double-blind trial Control: xanthan	Improvement of joint pain, increase of mobility and reduction of dependency to analgesics in patients consuming CH dietary supplement	8

Moskowitz *et al.*, 2000[56]	CH		10 g/day ID: 24 weeks + 8 weeks post-treatment washout	Patients with knee OA (Germany, UK, US) Y: 45–81 N = 389	Multicenter, prospective, randomized, double-blind, placebo-controlled trial	No statistically significant improvement in Western Ontario and McMaster Universities (WOMAC) pain score, function score and patient global assessment for the total study group Pain reduction and functional improvement in German patients but not in global patient evaluation Tendency to be more effective in severe OA (*post-hoc* analysis)	9
Zuckley *et al.*, 2004[55]	CH		10 g/day with calcium (300 mg/day) and vitamin C (60 mg/day) ID: 14 weeks	Patients with symptoms of mild knee OAMean y: 57N = 190	Randomized, double-blind, placebo-controlled trial	Improvement of certain strength and work performance tests (improvement of knee functional mobility on isometric and isokinetic testings) Greater effects on patients with more severe OA	
Benito-Ruiz *et al.*, 2009[57]	CH		10 g/day ID: 6 months	Patients with primary OA Mean y: 59 N = 250	Randomized, double-blind, multicenter controlled trial	Improvement of knee joint comfort as assessed by VAS and WOMAC	13

Polyphenols

Belcaro *et al*, 2008[75]	Pycnogenol®		NP ID: 3 months	OA patients with elevated C-reactive protein and plasma-free radicals N = 35	Placebo-controlled trial	Decrease of plasma-free radicals compared to baseline Decrease of plasma C-reactive protein level compared to baseline and placebo group Decrease of fibrinogen level compared to baseline No difference for plasma-free radicals, C-reactive protein and fibrinogen in placebo group Decrease of systemic inflammatory markers	
Cisar *et al*, 2008[72]	Pycnogenol®		150 mg/day ID: 3 months	Slovakian patients with mild to moderate OA Y: 25–65 N = 100	Prospective, double-blind, placebo-controlled, single centre study	Improvement of WOMAC index (pain, stiffness and global score compared to both baseline and placebo group, physical function compared to baseline only) and alleviation of pain by VAS compared to placebo Diminution of analgesic use compared to baseline and increase use in placebo group	13
Belcaro *et al*, 2008[76]	Pycnogenol®		100 mg/day ID: 3 months	Patients with knee OA and disability Mean y: 47.6 –48.6 N = 156	Double-blind, placebo-controlled randomized trial	Improvement of WOMAC index (pain, stiffness, physical function and global scores), well-being of patients and social functions compared to the beginning and to placebo group Increase of patient performance Decrease of oedema Decrease of analgesic (NSAID) medication compared to the beginning and to placebo group	11
Farid *et al*, 2007[74]	Pycnogenol®		150 mg/day ID: 3 months	Patients with knee OA Y: 25–65 N = 37	Double-blind, placebo-controlled randomized trial with parallel-group design	Improvement of WOMAC index (pain, physical function and global score but not stiffness) from second month compared to baseline and placebo Reduced use of medication (NSAID and COX-2 inhibitor) compared to placebo	11

Table VII
Summary of scientific consensus on the reviewed ingredients

Ingredient	Scientific consensus
n-3 PUFA	Lack of evidence of efficacy
ASU	Good evidence of efficacy
CH	Limited evidence of efficacy but suggestive
Vitamin D	Limited evidence of efficacy but suggestive
Nobiletin	Lack of evidence of efficacy
Curcumin	Preclinical evidence of efficacy
Genistein	Lack of evidence of efficacy
EGCG	Preclinical evidence of efficacy
Resveratrol	Preclinical evidence of efficacy
Pycnogenol	Moderate evidence of efficacy
Probiotics	Lack of evidence of efficacy

release, MMP-3, MMP-13, and PGE$_2$ production in normal chondrocytes[31,33,34,36]. It was also shown that ASU mixture contained in Piascledine®300 restored aggrecan production and inhibited MMP-3 synthesis in OA chondrocytes stimulated with IL-1β[35]. Other ASU

formulations produced similar effect on IL-1β, COX-2 and iNOS in LPS-stimulated chondrocytes[37] and were also shown to enhance transforming growth factor (TGF)-β production in bovine articular chondrocytes[38]. The ASU contained in Piascledine®300 were also shown to have beneficial effects on osteoblasts and synoviocytes. Indeed, this compound prevented inhibitory effect of osteoblasts on chondrocyte matrix component synthesis[39] and reversed IL-1β-induced collagenase production by synoviocytes[33].

Four in vivo studies with ASU were identified. They were performed in four different animal models of OA and they all support the beneficial effect of Piascledine®300 in OA. Piascledine®300 treatment prevents cartilage degradation (decrease in cartilage lesion severity, increase in cartilage thickness) by stimulating matrix component production as glycosaminoglycans (GAG) and PGs content in experimental models[27,28,40]. In addition, one in vivo study in dogs without any diagnosed joint disease suggests an effect of ASU by increasing growth factors (TGF-β1 and 2) involved in extracellular matrix synthesis[41]. All these preclinical studies strongly support anti-OA properties for ASU mixtures.

Table VIII
Summary of the ongoing CTs for the studied products on OA

Product	Population	Design	Primary outcome measure	Sponsor	Stage
ASU (Piascledin® 300)	Patients with hip OA Age: 45–75 years Enrollment: NP	Multicenter, randomized, double-blind, placebo-controlled trial Duration: 3 years Treatment: Piascledine® 300	Effect of treatment on joint space narrowing evaluated on X-ray	Laboratoires Expanscience	Completed
CH solution 10 g/day	Patients with knee OA Age: 49–90 years Enrollment: 30	Treatment, randomized, double-blind (subject, caregiver, investigator, outcome assessors), placebo-controlled, parallel assignment, safety-efficacy study	Effect of collagen hydrolysis on knee cartilage measured by MRI	GELITA	Completed
Vitamin D (cholecalciferol) 2 000 UI/day (capsule)	Patients with symptomatic knee OA Age: 45–90 years Enrollment: 146	Treatment, randomized, double-blind (subject, caregiver, investigator, outcomes assessor), parallel assignment, efficacy study Duration: 2 years Treatments: vitamin D3 2 000 UI daily or placebo	Cartilage volume loss (MRI) Knee symptoms (WOMAC)	National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) Office of Dietary Supplement (ODS)	Ongoing, not recruiting
Vitamin D (cholecalciferol) 2 000 UI/day (capsule)	Patients undergoing uni-lateral total knee replacement due to sever OA Age: 60 years Enrollment: 80	Treatment, randomized, double-blind (subject, caregiver, investigator, outcomes assessor), dose comparison, parallel assignment, efficacy study Duration: 2 years Treatments: vitamin D3 2 000 UI or 800 UI daily	Pain and function of the operated and non-operated knee Rate of falls	University of Zurich Harvard School of Public Health Tufts University Boston University	Recruiting
Curcuma domestica 1 500 mg/day (oral) divided into 3 times for 28 days	Patients with knee OA Age: 50–75 years Enrollment: 396	Treatment, randomized, double-blind (subject, outcomes assessor), active control, parallel assignment, safety/efficacy study Duration: 28 days Treatments: curcuma domestica or ibuprofen (1 200 mg/day)	Change in mean WOMAC pain scale	Mahidol University National research Council of Thailand	Not yet open for patient recruitment
Highly bioavailable turmeric extract (Arantal®) four capsules/day	Patients with knee OA Age: 40–80 years Enrollment: 280	Treatment, randomized, placebo-controlled, double-blind, parallel assignment, efficacy/tolerance study Duration: 15 days Treatment: turmeric extract (Arantal®) or placebo	Pain assessment using VAS	Bioxtract S.A. NuKleus	Recruiting

Clinical data

The results of four CTs, all double-blind placebo-controlled and randomized, were published and identified in the literature. All of these studies used the same pharmaceutical product, Piascledine®300, which is under drug authorization (AMM). All of them investigated the beneficial effects of Piascledine®300 on patients with symptomatic OA. All of them were conducted in patients with primary knee or hip OA and were by the way focusing on OA treatment. OA outcomes were NSAID/analgesics medication replacement, pain, function and structural changes (joint space narrowing). They studied the potential of symptom-modifying effect of Piascledine®300 and one of them was interested in the structure-modifying effect of ASU. Three out of the four evaluated studies were of good methodological quality (Lequesne et al, 2002[42], score: 11.5; Appelboom et al, 2001[43], score: 11; Maheu et al, 1998[44], score: 14).

The only one study assessing efficacy of Piascledine®300 in modifying articular structure failed to show any structural effect in patients with hip OA in spite of its long duration (2 years)[42]. However, a subgroup analysis suggested an effect in patients with the most severe hip OA, supporting further studies in this population group.

Data suggested that Piascledine®300 decreased NSAID/analgesic intake in the medium term (3–6 months) for patients with hip or knee OA[43–45]. However, the only long-term study (2 years) did not show any effect of Piascledine®300 on the NSAID consumption in patients with hip OA[42]. Nevertheless, the later was designed to detect radiographic changes more than to study the changes in symptoms. This could explain the fact that no difference was observed on clinical parameters (function, pain and NSAIDs consumption). In contrast, data regarding pain and patient's global assessment are more conflicting[42–45]. Piascledine®300 treatment seemed to improve patient's function assessed by LFI (Lequesne Functional Index) or VAS (Visual Analog Scale)[43–45].

A recent meta-analysis evaluating these four clinical studies concluded Piascledine®300 was efficient for reducing pain and improving function in OA[46].

Collagen hydrosylates (CHs)

CH is obtained by the enzymatic hydrolysis of collagenous tissues (bone, hide or hide split) form mammals. The main characteristic of CH is its amino acid composition, which is identical to type II collagen, thus providing high levels of glycine and proline, two amino acids essential for the stability and regeneration of cartilage[47]. This product is generally recognized as a safe food ingredient by regulatory agencies. CH is well digested and is preferentially accumulated in cartilage[48]. Although clinical use of CH is associated with minimal adverse effects, some gastro-intestinal side effects, as fullness and unpleasant taste, have been described.

In most studies, CH was administered alone in a water solution. However, it seems that CH is well absorbed and digested in other food matrix, such as fermented milk[47].

In vitro and preclinical data

Few preclinical data on the effect of CH on OA were identified in the literature. The search retrieved only one in vitro study assessing the stimulation of articular cartilage matrix by CH in cultured bovine chondrocytes[49]. This study demonstrated the stimulatory potency of CH on type II collagen and PG synthesis, as well as aggrecan expression by chondrocytes. This result was also reported in a scientific communication on CH effect in porcine chondrocytes[50]. In addition, an in vivo study with STR/ort mice which spontaneously developed OA[51] has shown that long-term CH supplementation may decrease OA cartilage degeneration and delay the progression of OA. These results are in favor of a disease-modifying effect of CH and its potential efficacy in OA. The

chondroprotective effect of CH was also confirmed in an other study investigating both in vitro and in vivo effect in mice[52]. CH was demonstrated to protect cartilage against degradation induced by phosphorus injection. The same study showed that CH prevented chondrocyte differentiation into mineralized chondrocytes.

Clinical data

Only three relevant CTs were identified in the literature. The first study investigated the effect of CH supplementation in healthy adult without degenerative joint disease but with joint pain. This study of medium methodological quality (score: 8)[53] showed that CH dietary supplement can improve joint pain, mobility and reduce analgesic medication in healthy active adults without degenerative joint disease. The CH supplementation can then improve knee function during joint-stressing activities. These observations were also reported in a scientific communication[54] in patients with symptomatic mild OA patients. The second report of medium methodological quality (score: 9)[55] mentioned a better effect of CH compared to placebo in severe OA patients than in the overall studied population. More recently, in another relevant CT of very good methodological quality (score: 13), the joint function improvement after CH treatment was shown in patients with primary OA[56].

Vitamin D

OA was traditionally considered as a cartilage disease, characterized by cartilage degeneration. But many evidences, as osteophytosis, subchondral bone sclerosis and cyst formation have grown up and demonstrated the prominent role played by subchondral bone in OA pathophysiology[57]. Some studies even indicate that bone alteration could precede cartilage changes[58]. But whether bone abnormalities, such as bone sclerosis, initiate or are simply involved in the progression of cartilage degradation is under discussion[59–61].

Normal bone metabolism depends on the presence of vitamin D, a compound derived mostly from cutaneous exposure to ultraviolet and from the diet in a lesser extent. Suboptimal levels of vitamin D may have adverse effects on calcium metabolism, osteoblastic activity, matrix ossification and bone density. Low serum levels of vitamin D may increase the progression of knee OA[62] and may impair the ability of bone to respond optimally to OA pathophysiologic processes and may predispose patients to joint degradation[57]. High prevalence of low vitamin D status has been demonstrated in persons with knee OA[63]. Moreover, in low bone mineral density (BMD) patient, the level of vitamin D seemed to influence the incidence and progression of the disease[62]. Some studies have consequently investigated the relationship between vitamin D and OA. The main drawback is that they are all observational. Most of them have looked at the association of vitamin D serum level rather than vitamin D intake with OA.

In vitro and preclinical data

Three in vitro studies investigating the role of 1,25-dihydroxy-vitamin D3 in the pathophysiology of OA are available[59,64,65]. These studies used different cell models: synovial fibroblasts, chondrocytes or osteoblasts. Data showed that 1,25-dihydroxyvitamin D3 had no inhibitory effect on articular catabolic enzymes and on a potent pro-inflammatory mediators but rather upregulated catabolic enzymes (MMP-1 and -3) in human articular chondrocytes[64,65]. Interestingly, 1,25-dihydroxyvitamin D3 exerts a positive effect on rheumatoid synovial fibroblasts by reducing MMP and PGE_2 production[65]. In addition, 1,25-dihydroxyvitamin D3-induced osteocalcin production appears to be increased in OA osteoblasts compared to healthy one, which can cause an increase

in bone metabolism resulting in bone sclerosis and osteophyte formation[59].

Only one *in vivo* study was identified. This study evaluated the impact of a supplementation of vitamin D3 (25-hydroxyvitamin D3) on OA in pigs[66]. The supplementation revealed no effect on the incidence or the severity of OA lesions, articular PG and collagen content.

Clinical data

The majority of available evidence on the efficacy of vitamin D in the treatment or in the prevention of OA comes from epidemiological data. Most of them investigated the relationship between vitamin D serum levels and joint structure parameters (joint space narrowing, osteophytes, cartilage loss and volume). A total of four studies have assessed this relationship[57,67,68] and only one has investigated the association of vitamin D intake and OA[57]. This study revealed that low levels of vitamin D intake are related to an increased risk of OA progression but no correlation was made with OA incidence. Interestingly, in this study, vitamin D intake modestly correlated with vitamin D serum levels. In addition, one other study was found to evaluate the prevalence of vitamin D deficiency in individuals with OA[63]. This study revealed a high prevalence of low vitamin D status in patients with knee OA. The other studies investigating the association between vitamin D serum level (25-vitamin D) and joint structure parameters gave inconsistent data. Two of them showed a relationship between vitamin D serum level and OA parameters such as joint space narrowing and/or cartilage volume in the knee or hip joint[67,69]. An other study did not show any association between vitamin D serum level and joint space loss in hip OA patients[68]. This difference could be explained by the fact that the population of this study was younger than the population of the other two. However no linear association was found when OA was assessed with a structural global score[57,70]. These studies suggested a U-shaped relationship. Serum Vitamin D level is reported to be predictive of knee OA, when measured by quartiles with the lowest risk in the middle quartile[70].

Polyphenols

Research on the effect of dietary polyphenols on human health has developed considerably in the past 10 years. The results strongly support the role of polyphenols in the prevention of degenerative diseases. The anti-oxidant properties of polyphenols have been widely studied, but it has become clear that the mechanisms of action go beyond the modulation of oxidative stress. Some researchers have investigated the potential effect of some polyphenols in OA. Only pine bark extract-Pycnogenol® has been tested in CTs.

Pine bark extract-Pycnogenol®

Pycnogenol® is a special standardized extract from the bark of the French maritime pine (*Pinus pinaster*). This extract represents a concentrate of polyphenols, containing several phenolic acids, catechin, taxifolin and procyanidins with various biological and clinical effects[71]. No-preclinical or *in vitro* data were found for Pycnogenol® and OA. However, the anti-oxidant and anti-inflammatory profile of Pycnogenol® and its inhibitory effect on MMPs and iNOS are well documented in conditions other than OA[72,73].

The symptom-modifying effect of Pycnogenol® has been relatively well documented in OA patients. The search retrieved four CTs [71,73–75]. They assessed the impact of Pycnogenol® in patients with knee OA in a medium term (3 months). These studies indicate that the daily intake of 150 mg of Pycnogenol® alleviated OA symptoms. All studies showed that Pycnogenol® was effective in reducing NSAIDs or COX-2 inhibitor medication, suggesting that Pycnogenol® could be used as an effective adjuvant treatment. Data strongly support the pain-alleviating effect of Pycnogenol®. Despite some discordant results, physical function and stiffness seem to be improved by intake of Pycnogenol® in OA subjects. All these studies are of good to very good quality (Farid *et al*, 2007[73] and Belcaro *et al*, 2008[75], score: 11; Cisar *et al*, 2008[71], score: 13).

Nobiletin

Nobiletin is a citrus polymethoxyflavone which was proven to have pharmacological actions as anti-inflammatory, anti-tumor proliferation and anti-tumor invasion and metastasis *in vitro* and *in vivo*[76]. This product has been exclusively studied *in vitro* in synovial fibroblasts and in articular chondrocytes. Nobiletin was able to inhibit the production of catabolic factors (MMP-3 and -9, ADAMTS-4 and 5) and of mediators of inflammation (PGE_2) in rabbit and human synovial fibroblasts[76–78]. Nobiletin was also showed to activate the MMP inhibitor (TIMP-1)[78]. This inhibitory potential was also demonstrated in rabbit articular chondrocytes[77]. Nobiletin demonstrated a potential to inhibit cartilage degradation. This chondroprotective potency should be further documented.

Curcumin

Curcumin (diferuloylmethane) is the major component of turmeric, a yellow spice derived from the plant *Curcuma longa* and a potent anti-oxidant. It has been extensively investigated due to its anti-tumor, anti-oxidant and anti-inflammatory and analgesic properties. We have recently reviewed the biological activities of curcumin[79]. The anti-OA potential of curcumin has been widely studied *in vitro*. Twelve studies were found. They were all carried out in chondrocytes or on articular cartilage explants. Curcumin was able to downregulate catabolic and degradative effect observed in cartilage explants or chondrocytes stimulated with IL-1β, LPS or tumor necrosis factor (TNF). Curcumin inhibited the production of MMP-3, -9 and -13[80–82] and restored type II collagen and GAG synthesis[81–85]. Curcumin positive effect on GAG release was confirmed[86]. In addition, curcumin demonstrated potent anti-inflammatory properties by inhibiting key inflammatory mediators (IL-6, IL-8, PGE_2, NO) and enzymes (COX-2 and iNOS)[87–90] and anti-catabolic properties by inhibiting MMP-3 synthesis[89]. Curcumin has also demonstrated anti-apoptotic activity on chondrocytes[83] and growth-inhibitory and pro-apoptotic effects on synovial adherent cells, which are the main source of inflammatory mediators and other mediators of cartilage degradation, all of them playing key role in the pathogenesis of arthritis[91]. This is important to note that one study has reported a toxic effect of curcumin used at high dosage (50 μM) without any beneficial effect in cartilage matrix[84]. This study was performed in a novel immortalized human OA chondrocytes model, which can explain the discordance with previous studies.

No clinical data are available for the effect of curcumin in OA. However, one study tested the clinical efficacy of a herbomineral formulation containing a component rich in curcumin in subjects with OA in a randomized, double-blind, placebo-controlled, cross-over study[92]. Positive results in pain management and mobility were obtained in the treated group. Curcumin in OA is a current research interest.

Genistein

Genistein is one of the several known isoflavones and is found in soybeans and soy products. Genistein is considered as a phytoestrogen. Clinical observations have suggested a relationship between OA and a changed estrogen metabolism in menopausal

women. Moreover phytoestrogens have been shown to ameliorate various menopausal symptoms[93]. The effect of phytoestrogens, including genistein, has been studied on articular cartilage matrix metabolism and inflammation. Nevertheless, the data for genistein and OA are limited. The *in vitro* and preclinical data are not consistent to support a beneficial effect of genistein on articular cartilage. Genistein does not affect cartilage metabolism[93,94] but could have an anti-inflammatory effect by suppressing COX-2 but not NO production[94]. In addition, the consumption of an extract of soy phytoestrogen in animal failed to modify cartilage metabolism in ovariectomised monkey[95]. Additional experiments are needed to clarify the potential benefit of genistein in articular cartilage metabolism.

Epigallocatechin-3-Gallate (EGCG)

EGCG is a major component of the polyphenolic fraction of green tea and exhibits anti-oxidant, anti-tumor and anti-mutagenic activities. The *in vitro* effect of EGCG is well documented. Most of the available data on EGCG and OA come from experiments performed *in vitro* in human chondrocytes looking at the anti-inflammatory effect of EGCG. Data suggest that EGCG exerts an anti-inflammatory effect on OA chondrocytes by inhibiting the production of key inflammatory mediators (NO, PGE_2, COX-2 and iNOS)[96–98]. This anti-inflammatory effect has also been observed in osteoblasts by the inhibition of IL-6[99] and synovial fibroblasts by the inhibition of COX-2 expression and synthesis and by the inhibition of PGE_2 and IL-8 secretion[100]. Additional anti-inflammatory and anti-catabolic effects have been demonstrated for EGCG in human chondrocytes. Indeed, EGCG can inhibit the TNF-α and MMP-13 production induced by advanced glycation end products (AGEs) which are responsible for cartilage mechanical properties loss[101]. This effect could happen through the attenuation of MAP kinase activation and inhibition of nuclear factor κB (NF-κB) activation. This is supported by the previous report of anti-catabolic activity and inhibitory effect on NF-κB and AP-1 signalling[102]. Finally, this is of interest to note that the polyphenolic fraction of green tea can prevent the onset of arthritis and the severity of the disease in mice collagen-induced arthritis[103]. Complementary experiments are necessary in order to confirm the anti-inflammatory effect of EGCG on OA *in vivo*.

Resveratrol

Resveratrol is a stilbene that is naturally present at high concentration in grape skin and red wine. It has significant anti-inflammatory and anti-oxidant properties which could be beneficial in OA[104]. Only *in vitro* studies were identified for this compound. A total of five studies were performed in cartilage explants and chondrocytes[85,104–107]. These studies indicated that resveratrol can have beneficial effects. It has demonstrated anti-inflammatory and anti-apoptotic properties[104,106]. Resveratrol inhibited catabolic factors as MMPs and pro-inflammatory mediators as PGE_2 and COX-2, and stimulated the synthesis of matrix components (PG, GAG, type II collagen)[104,106,107]. These effects could prevent cartilage degradation. In addition, cartilage protection may be achieved with intra-articular injection of resveratrol. This was observed both in anterior cruciate ligament transaction OA model and LPS-induced arthritis model in rabbit[108,109]. These preclinical evaluations indicate an interesting potential of resveratrol in OA but additional *in vivo* studies are needed.

Prodelphinidins

Prodelphinidins are a type of condensed tannins and are composed of gallocatechin and epigallocathechin[110]. Only one *in vitro* study has investigated the effect of prodelphinidins. This study showed potential *in vitro* effects on human chondrocytes. Prodelphinidins seemed to increase PG and type II collagen and inhibited PGE_2 synthesis by acting on COX-2[110]. These data are in favor of additional preclinical evaluations.

Quercetin

Quercetin is a plant-derived flavonoid. Anti-inflammatory and anti-oxidant properties have been suggested for this nutraceutical. Only one *in vitro* study in human synovial cells has been identified[111]. This study demonstrated potential anti-inflammatory effect by the inhibition of TNF-α mediated-IL-8 and monocyte chemo-attractant protein-1 (MCP-1) expression. This suggested a potential anti-arthritic effect but further preclinical investigations are needed especially in OA.

Ventol

Ventol is a phlorotannin-rich natural agent derived from *Ecklonia cava* with anti-oxidant and anti-inflammatory activities[112]. One *in vitro* study on cartilage explants has shown the inhibition of PG degradation after IL-1α stimulation. This should be further studied to confirm the anti-OA potential of this compound.

Discussion

OA is a debiliting chronic disease with a serious need of alternative treatments that could help patients to preserve their joint function and therefore maintain a certain quality of life. We have here summarized most of the published effects of some nutraceuticals. Many results have been highlighted and the quality of the studies addressed.

Globally, we can conclude to a lack of evidence for most of the studied compounds. The review by *Ameye and Chee*[6] has also analyzed the available studies at that time for ASU, n-3 PUFAs and CH. The same conclusions as ours were made, meaning that there are no strong clinical data available. So, there is a need for additional preclinical studies and CTs of good quality. In addition, this is important to note that the potencies demonstrated in preclinical studies for most compounds are not concordant with their clinical efficacy. This could be explained by the doses used *in vitro* and in animal models that are most of the time higher that the ones used in CTs. An other explanation could come from the fact that preclinical studies deal most of the time with early stages of the disease whereas CTs involve patients at later stages. This can justify the discordance between preclinical data and clinical and epidemiological observations.

Many efforts have been carried out to find a cure for OA. OA management is a challenge for physicians and rheumatologists. Many alternatives are now available but recommendations have to be done. Nutraceuticals is one of these alternatives. They have great potential in OA but there is a need of strongly substantiated data.

Many questions still remain to be addressed. Where should be the line between food and drugs drawn? This issue is well illustrated by ASU mixture. ASU are considered as prescribed drugs in France, but as food supplement in other countries. The prescribed drug, Piascledine®300, is now recommended for the treatment of OA symptoms. These recommendations are based on strong clinical evidence. However, *in vitro* studies have demonstrated that some of the beneficial effects of Piascledine®300 were related to its particular formulation resulting of a patented extraction process, and were not observed with other formulation (personal communication). This extraction process is responsible for specific changes in the unsaponifiable fractions of avocado and soybean that could

explain their particular effects. This means that the natural food ingredient has underwent some modifications that are related to particular biological effects. Therefore, ASU should be chemically analyzed before to be considered as a food supplement or a nutraceutical. Nutraceutical is a broad term to qualify any product derived from food source that provides extra health benefits. This definition fails to precise whether a modified product derived from food source can be considered as a nutraceutical. The line between a nutraceutical and a drug is thin. Other criterion that can help to distinguish between a drug, food supplement, and nutraceuticals are the oral dose administrated and the bioavailability. Food supplement should be administrated at concentration found in the normal diet. Bioavailability would be a great criterion to demonstrate the potent efficacy of a product. For example, the pharmacokinetic of curcumin has been studied[113] and many parameters that are opposed to curcumin efficacy have been described. In human, the serum concentrations of curcumin after an oral massive dose of 4, 6 or 8 g/day reach 0.5, 0.6 and 1.8 μM respectively. These concentrations are below the concentration showing a biological activity *in vitro*. However, new formulation of curcumin[114] has enhanced cellular uptake, increased bioactivity *in vitro* and improved bioavailability *in vivo*. This is in favor of a better efficacy, meaning that the anti-inflammatory and other potencies of curcumin could be even more effective. Curcumin would become by the way a potent anti-inflammatory agent. For this reason, a clinical study has been initiated with complexed curcumin in patients with knee OA. This complexed curcumin (Arantal®) showed a 7000 times increased solubility. Can it be considered as a food supplement any longer? This critical point can also be illustrated by resveratrol. The pharmacokinetic of resveratrol can be modified by the addition of glycosyl groups that improved its absorption[115]. This process could be applied to other polyphenols to increase their bioavailability.

Can nutraceuticals still be considered as food supplement when used under drug formulation? These modifications should also be controlled and regulated. Moreover, food nutrients can target multiple pathways compared to monomodal mode of action of drugs. For example, some natural anti-oxidant peptides have also been identified in porcine CH[116]. CH has hypotensive potential[117,118]. This is an additional advantage for the use of CH. Moreover, it has recently been demonstrated that some nutraceuticals are even more effective when used in combination. It has been shown for EGCG and ASU[119] and resveratrol and curcumin[120]. Safety and toxicity of nutraceuticals added alone or in combination should also be addressed, especially if bioavailability and biological activity are increased. Pharmacokinetic of all these compounds should be described and safety monitoring should be done.

For these reasons, the safety and effectiveness of these products are under strict regulation in Europe. The problem is that the criterion of evaluation for clinical studies are defined as for pharmaceutic standards and this penalizes foods in terms of effect size and also of absence of acute effect. OA as a chronic disease has a long time window for intervention and should benefit from functional food alternatives to pharmacological interventions to improve subjects' quality of life day after day.

We have also searched for the ongoing CTs with nutraceuticals. They are all summarized on Table VIII and given an overview of the current research interest on the selected ingredients. The number of ongoing trials supports the growing interest to identify their role in OA treatment. Two CTs are ongoing in order to evaluate the efficacy of vitamin D supplementation on pain, mobility and joint structure in OA patients. Because of their high quality (long duration and strong study design) these results will be valuable for the determination of the role that could play vitamin D supplementation in OA. More evidences are needed regarding the role that it

could play in the prevention of OA. Two CTs are ongoing on curcumin to determine whether the consumption of a plant extract with high curcumin content alleviates pain in subjects with OA. Furthermore, one CT was just completed with CH. It was the first one interested in the structure-modifying effect of the studied compound. The results are not yet available. Additional CTs should assess the potential of nutraceuticals in a long-term use in OA and should also be interested in their structure-modifying effect which is the great challenge in seeking new OA treatment.

We have shown here that most of the studied compounds could have beneficial effects in the treatment of OA, even if we still need more evidence. They could represent great alternatives for OA management. We have only detailed here the information for a few compounds but others can also represent great potential. Indeed, other lipids, vitamins, minerals or plant extracts have also been demonstrated to be effective in OA. Probiotics could also be considered for the treatment of OA. Several studies have highlighted the importance of intestinal flora in inflammatory auto-immune diseases, such as rheumatoid arthritis. Beneficial anti-inflammatory properties and modulatory effect of immune response of probiotics on rheumatoid arthritis were demonstrated in animals[121–123]. In addition, the consumption of lactobacilli contained in a yogurt demonstrated the preventive and curative effects on T-cell-dependent experimental arthritis by reducing the intensity of inflammation and joint destruction in animals[124]. In the same way, CTs have evaluated the effects of probiotics on the activity and on the symptoms of rheumatoid arthritis[125,126]. The positive findings of these studies suggested the need of complementary studies on the effects on probiotic bacteria in rheumatoid arthritis. There is a lack of data on the effect of probiotics on OA in the literature. The potential effect of probiotics on OA has not been extensively studied but the data on their effect in arthritis suggest that they could have a potential interest in OA.

Conclusion

We have gathered here all the studies and CTs about some nutraceuticals. Some results are really promising and encouraging. The main concern remains the quality of studies. There is a need of serious and well designed studies that could answer most of the questions regarding the safety and efficacy of such compounds. This could help their recommendation for OA treatment.

Many studies have been carried out in order to highlight the potency of several nutraceuticals for the treatment of OA. Nutraceuticals offer a large variety of products with a wide range of effects. They open new and large horizons for the treatment of chronic disease as OA. Clinical endpoints for foods should be reconsidered. Further investigations are needed but nutraceuticals are not negligible for OA management. They should be considered as potent adjuvant treatments with NSAIDs for example. Early markers development would also enable to build prevention strategies for food, in absence of cure treatment for OA.

Author contributions

Conception and design of the study: YH, EC.
Acquisition of data: CL, DC, CR.
Analysis and interpretation of data: YH, EC, CL, DC, CR.
Preparation of manuscript: YH, EC, DC.
Final approval of manuscript: YH, CL, DC, CR, EC.

Conflict of interest

YH is a consultant for BioXtract and Danone. He is also the coordinator of the CTs on curcuma operated by BioXtract. (Arantal®; NCT00992004). Naturalpha was financed by Danone to set-up

scoring methodology, perform the identification, selection and scoring of relevant studies for the review. Other authors declare no conflict of interest.

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