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Nutraceuticals in joint health: animal models as instrumental tools

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Osteoarthritis (OA) is a degenerative joint disease with no curative treatments. Many studies have begun to demonstrate the efficacy of nutraceuticals for slowing down OA. Animal models are utilized as a compulsory step in demonstrating the protective potential of these compounds on joint health. Nevertheless, there exist a wide variety of available OA models and selecting a suitable system for evaluating the effects of a specific compound remains difficult. Here, we discuss animal studies that have investigated nutraceutical effects on OA. In particular, we highlight the large spectrum of animal models that are currently accepted for examining the OA-related effects of nutraceuticals, giving recommendations for their use.

Introduction

OA is a chronically evolving degenerative disease that affects a growing number of individuals within our aging population, and is associated with both a higher risk of comorbidity [1] and a strong socio-economic burden [2]. The World Health Organization (WHO) reported in 2003 that approximately 10% of humans over 60-years old displayed significant clinical symptoms of OA, with >50% of humans over the age of 65 showing radiological evidence of OA. Moreover, it is predicted that the prevalence of OA will continue to increase in coming years as the population ages, especially if preventive measures are not taken.

From a physiopathological viewpoint, OA is a heterogeneous disease that induces whole-joint damage [3]. It is characterized by progressive cartilage loss, subchondral bone remodeling, osteophyte formation and a low-grade inflammation that affects all joint tissues, including the synovium and the infrapatellar fat pad (IFP) (Fig. 1).

OA begins with re-entry of articular chondrocytes into a proliferative program, leading to the formation of clusters with increased anabolic activity and upregulated extracellular matrix synthesis [4]. Eventually, these cells acquire pathological features, becoming hypertrophic repair-like chondrocytes that display augmented catabolic activity. These hypertrophic chondrocytes, along with synovial and immune cells, secrete prostaglandins and interleukin 1beta (IL1β), which contribute to increased cartilage catabolism. Ultimately, metabolic homeostasis is then broken and the degradation of matrix products overwhelms biosynthesis. This phenomenon primarily results from the synthesis of matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), which is associated with a decrease in the tissue inhibitor of metalloproteinases (TIMPs) [4]. In addition, overexpressed cytokines lead to an increased production of nitric oxide (NO), prostaglandin E2 (PGE2) and leukotrienes, which eventually promote chondrocyte

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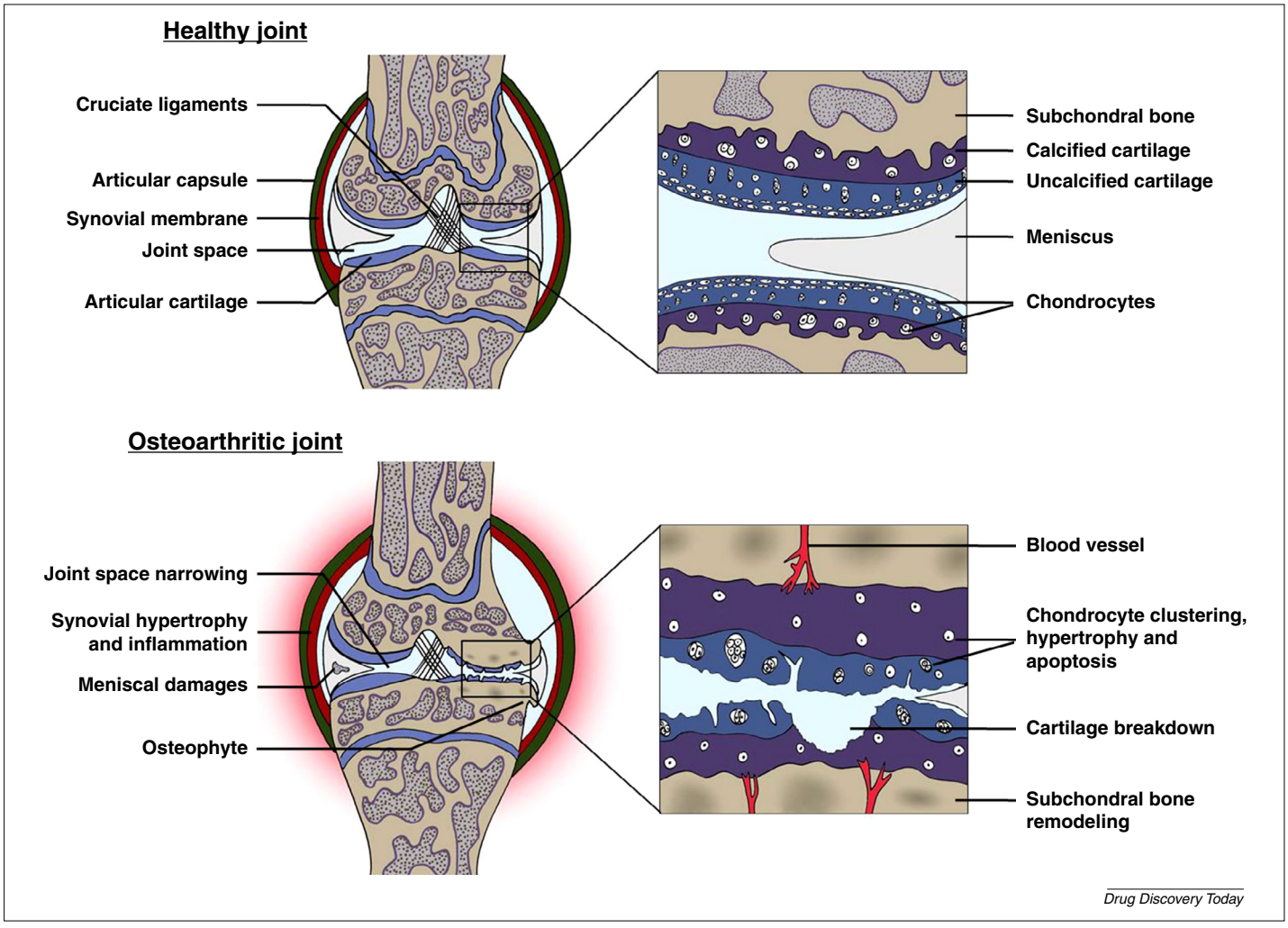

FIGURE 1

Joint tissue affections in knee osteoarthritis (OA). Osteoarthritic joints are characterized by a loss of articular cartilage, synovitis and synovial hypertrophy, as well as sclerosis of subchondral bone, which is accompanied by formation of osteophytes at the joint margins.

apoptosis [5]. In other joint tissues, histological and cellular disorders can also contribute to altered joint homeostasis [3]. Early changes include hyperplasia of the synovial cells ('synovitis'), muscle weakness [6] and fibrillation of the meniscal tissue, as well as thickening of both subchondral bone plate and epiphyseal spongiosa, which are associated with decreased bone mineral content [7].

Collectively, the aforementioned events lead to advanced OA, in which articular cartilage loss is initiated via fibrillation of the superficial zone followed by cartilage damage. Furthermore, cartilage matrix degradation products that are released into the synovial fluid exacerbate inflammation, which results in infiltration of innate immune cells into joint tissues (synovium and IFP) and secretion of inflammatory mediators. As the calcified cartilage zone thickens, the tidemark (i.e. the boundary between calcified and uncalcified articular cartilage) migrates towards the superficial zone [8]. Subsequently, the calcified cartilage becomes invaded by vascular elements, as seen during the endochondral ossification process [9]. Cartilage vascularization enables the recruitment of stem cells, which initiate repair processes (i.e. formation of fibrous cartilage) and lead to an increase in oxygen pressure.

Severe damage of articular cartilage leads to the eburnation of subchondral bone, which produces an imbalance in the mechanical loading of the joint surface. This is likely to contribute to the formation of osteophytes at the joint margins.

Current OA management

To date, there is no curative treatment for OA in humans. Therefore, clinical management focuses only on OA symptoms (e.g. pain and inflammation), using analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs). However, these approaches do not prevent the progression of cartilage degradation and can be associated with adverse effects [10]. Nonpharmacological management currently represents the first line of therapy for OA and includes approaches such as weight loss, education, physical therapy or thermal treatment. Nevertheless, when nonpharmacological treatments become insufficient, pharmacological therapies are used. In this regard, analgesics (e.g. acetaminophen), NSAIDs (e.g. cyclooxygenase and/or lipooxygenase inhibitors) and steroidal anti-inflammatories (SAI) are prescribed to reduce OA-related pain and improve mobility [11]. Ultimately, failure of nonsurgical strategies can lead to total knee and hip replacement [12]. For this reason, an active field of

research involves the identification of agents that might prevent, delay or reverse the pathology of OA. Indeed, the emerging role of nutraceuticals could hold promise in this area [13].

The nutraceutical approach to OA treatment

The term 'nutraceutical' combines the words 'nutrition' and 'pharmaceutical' [14]. Thus, in some cases, the term 'dietary supplement' can be used instead. Nutraceuticals is a broad term referring to substances isolated or extracted from food, animals or plants, that have a proven physiological or medical benefit [15].

Currently, there is increasing evidence to suggest that nutraceuticals show protective effects for many pathologies, including cardiovascular disease and cancer [16]. In addition, they have been proposed to promote the maintenance of bone and joint integrity and/or health [17]. So far, several epidemiological studies have investigated the relation between vitamin D serum levels and human joint structure, as well as the link between vitamin D intake and OA [17]. The latter study revealed a good correlation between low vitamin D serum levels and the presence of OA in the knee [18]. Also, glucosamine sulfate and chondroitin sulfate, which are essential components for the synthesis of the proteoglycan molecules of the cartilage extracellular matrix, were reported to be possible nutraceuticals for the prevention or the treatment of OA [19]. However, although it has become well recognized that nutraceuticals are capable of promoting or improving cartilage health, appropriate animal models are necessary for evaluating the effects of these agents. As previously reviewed by other authors, OA can develop naturally in some species (e.g. mouse and guinea pig) or be induced through surgery or chemical treatments [20,21]. Therefore, it is currently necessary to delineate the most relevant and standardized animal models that will allow indepth investigation of the health benefits and modes of action of nutraceuticals in the management of OA.

Animal models used to study nutraceutical-based therapies for OA

Many animal species (e.g. mouse, rat, rabbit, sheep, dog, cat and monkey) have been used to study the effects of nutraceuticals on OA, including surgically or chemically induced as well as, spontaneous models (Table 1). However, none of these models can perfectly mimic OA, because these animals differ physiopathologically from humans in terms of their joint anatomy, genetics, biomechanics and disease parameters (severity, localization and kinetics). Various reviews providing detailed comparisons of these different OA animal models have been published elsewhere [20,21]. Here, we focus on identifying the most appropriate animal models for investigating the potential protective roles of nutraceuticals in OA.

The OA-related effects of various nutraceutical families (e.g. polyphenols [22–26], vitamins [27,28], protein [29,30], alkaloid [31] and others [32–35]) have been mostly studied using surgically induced OA models. This is probably because the surgical protocols have a 100% incidence, display a shorter lesion onset time and reproduce the traumatic etiology of the human disease. Anterior cruciate ligament transection (ACLT) and menisectomy are the two most commonly used procedures for inducing OA in animals. Surprisingly, although destabilization of the medial meniscus (DMM) and the groove model can be used to surgically induce an OA phenotype that more closely resembles the human disease than achieved with ACLT, these methods are not currently used in nutraceutical research. This might be because surgery on small mammals is difficult to standardize. For this reason, chemically induced OA models have also been developed. Intra-articular injection of active molecules, such as cytokines [e.g. IL1β and tumor necrosis factor alpha (TNFα)] [36,37], enzymes (e.g. papain and collagenase) [42,43] or chemicals [e.g. mono-iodoacetate (MIA)] [38–41], has been shown to induce pathologic changes that resemble OA. This is especially true for the MIA, an inhibitor of aerobic glycolysis that elicits chondrocyte death and articular pain, which can be utilized to produce OA that progresses in a similar fashion to that seen in humans [21]. Notably, OA lesions induced through the injection of active molecules are time and dose dependent and can be reversed by the use of low doses. These models have commonly been used to elucidate the anti-inflammatory and catabolic activities of polyphenols [22–26] and alkaloid [31] in OA.

Traumatic and chemical inductions of OA are convenient experimental approaches that enable the study of disease progression from the point of inception. Thus, these methods enable the exploration of nutraceutical effects that range from prevention to elimination of the disease. However, these approaches might not be the most relevant, especially because the onset of OA in humans occurs with aging, which implies that the full spectrum of OA-associated phenotypic features might require time to develop. In this regard, it is known that specific strains of guinea pigs, mice and monkeys can develop spontaneous OA with age. Therefore, these models enable us to study the slow and 'natural' progression of the disease (Table 1). They more closely resemble the human pathology, and might be especially relevant for investigating the preventive potential of specific nutraceuticals on the incidence of OA. Nevertheless, there are few experimental studies that have been conducted using spontaneous models of OA, which might be attributed to the global cost associated with the duration of such methods (i.e. animal facility costs and the amount of food or extract required). Finally and closely linked to clinical OA, animal patients (e.g. dogs and cats) were also used as models to assess the effects of nutraceuticals in studies focusing on improvement of life quality [42–44].

Recommendations for nutraceutical studies

Administration routes of nutraceuticals

Notably, two administration routes have been commonly used for nutraceuticals: local intra-articular injection and oral administration via diet supplementation or feeding (Table 1). The former route ensures control over of the locally delivered dose of nutraceutical compared with the latter. Although intra-articular injection helps to overcome bioavailability issues that might arise because of the oral route, repeated injections increase the risk of infection. Also, when performed in rats [36,41,45–47] and rabbits [23–25,27,33], there is a limited volume that can be injected into the joint cavity (50 ml and 300 ml, respectively). By contrast, oral supplementation of nutraceutical represents a more physiological approach and can be managed autonomously by the patient. In animal studies, oral administration has been performed via gastric gavage [27,28,38–40] or wafers, as well as capsular and other galenic forms [22,29,30,32,35,42,48], which enable more rigorous

TABLE 1

Effects of nutraceuticals on OA in animal studies[a]

Animal model of OA	Nutraceutical	Origin	Administration route	Duration treatment	Dose	Main results	Refs
Surgically induced							
Menisectomy (mouse)	Procyanidin B3 (polyphenol: flavonol)	Grape seed	Orally	4 weeks (5 days/week), 5 days after surgery	1 mg/10 g body weight	↘Total Mankin score ↘Thickness of pseudocapsule ↘Chondrocyte cell death ↘iNOS expression	[22]
MCL and partial meniscectomy (rat)	Vitamin D3 (vitamin)	Fish oil, liver and milk	Orally	40 days + 4 days before surgery	4IU (100 ng) to 4000 IU (100 μg)/kg	↘OA-induced hypertrophy of femoral condyle ↘*TLR4*, *MMP3*, *TNFa* and *IL1b* gene expression during OA induction but not progression	[27]
Partial medial menisectomy (rabbit)	Fursultiamine (vitamin)	Seeds and yeast	Orally	8 weeks 3 days after surgery	100 mg/kg	↘Total Mankin score ↘Cartilage fibrillation ↘Chondrocyte disorganization ↘MMP1 level enhances chondroprotective effects of GH/CS	[28]
ACLT (rat)	Quercetin and glutamine (polyphenol: flavonol)	Vegetable (onion)	Intra-articular injection	4 weeks, twice a week	Quercetin 600 μM and glutamine 200 mM in 50 μL PBS	↘Total Mankin score ↗HSP70 and aggrecan expression ↘Severity of OA	[45]
(rabbit)	Tetrandrine (alkaloid)	Root of *Stephania tetrandraine*	Intra-articular injection	1 month after surgery weekly for 6 weeks	20 mg/L in 300 μL	↘Mankin score ↘Cartilage fissures and degradation ↘MMP (1;3;13) gene expression	[31]
	Glucosamine (GAG)	Shellfish	Orally	3 weeks after surgery for 8 weeks	100 mg/day	↘Total Mankin score ↗PG content	[32]
	CMC (carbohydrate)	Shellfish	Intra-articular injection	Injection at 1, 3 and 5 weeks after surgery	0.3 mL 2% CMC	↘Cartilage degradation ↘*MMP1* gene expression	[33]
	Chlorogenic acid (polyphenol: phenolic acid)	Sunflower seeds, flour, artichoke, coffee and bean	Intra-articular injection	Weekly for 6 weeks after surgery	20 μM in 300 μL	↘Total Mankin score ↘Chondrocyte disorganization ↘Superficial cartilage lesions ↘MMP (1;3;13) ↗TIMPs	[23]
	Resveratrol (polyphenol: stilbene)	Red wine, grape skin, peanut and blackberry	Intra-articular injection	5 weeks after surgery, daily for 2 weeks,	10 μmol/kg	↘OA score ↘Synovial inflammation	[24]
ACLT + medial meniscus menisectomy (rabbit)	Resveratrol (polyphenol: stilbene)	Red wine, grape skin, peanut and blackberry	Intra-articular injection	5 weeks after surgery, daily for 2 weeks	10–20 or 50 μmol/kg	↘Total Mankin score ↘NO release in synovial fluid ↗Cartilage surface ↘TUNEL staining	[25]
ACLT + partial meniscetomy (rabbit)	Glucosamine, CS and manganese ascorbate (GAG: mineral)	Shellfish (glucosamine), vegetables and fruit (manganese)	Orally	14 weeks	Diet supplemented with 2% Cosamin®DS (500 mg/g glucosamine, 400 mg/g CS, 76 mg/g manganese ascorbate)	↘Total modified Mankin score ↗GAG synthesis ↘Catabolic enzymes	[34]

ACLT (Dog)	ASU (protein)	Avocado and soybean	Orally	8 weeks	10 mg/kg/day	↘Total OA Cook score ↘Superficial cartilage fibrillation ↗Subchondral bone parameters ↘iNOS and MMP13 immunostaining	[29]
	BCD extract (multifamily)	*Brachystemma calycinum*	Orally	8 weeks	200 mg/kg/day	↘Total OA severity score ↘Depth of cartilage lesions ↘iNOS and MMP13 expression ↗Mechanical properties	[35]
Menisectomy (ovine)	ASU (protein)	Avocado and soybean	Orally	1 month after surgery for 3–6 months	900 mg/day (Piascledine300®)	↘Mankin score ↗Uncalcified cartilage thickness ↘Subchondral bone sclerosis ↗PG content	[30]
Ovariectomy (monkey)	Soy protein extract enriched in Genistein (polyphenol: flavonoid)	Soy	Orally	36 months	129 mg/day	↗Articular cartilage thickness ↘Calcified cartilage thickness ↘OA severity score ↗Type 2 collagen expression?	[26]
Chemically induced							
MIA injection (mouse)	Pomegranate juice	Pomegranate	Orally	Daily for 2 weeks	4, 10 or 20 ml/kg	↘OA severity score ↘Cartilage damage ↘Inflammation of synovial membrane	[38]
(rat)	GSPE (polyphenol: anthocyanin)	Grape seed	Orally	4 weeks, 3 times a week after OA induction	100 or 300 mg/kg	↘Pain ↘Osteoclast number in subchondral bone and bone alterations (sclerosis and osteophyte) ↘Total Mankin score ↗Cartilage organization ↘IL1β and MMP13 immunostaining ↘Chondrocyte loss and PG content	[39]
	Silymarin (polyphenol: flavonolignan)	*Carduus marianus* (milk thistle)	Orally	14 days after OA induction	50 mg/kg	↘Fibrillated cartilage surface ↘Inflammation (NO, IL1β) ↘Oxidative stress (malondialdehyde and lipid peroxidation)	[40]
	Capsaicin (alkaloid)	Chili pepper	Single intra-articular injection	14 days before OA induction	0.5% in 100 μL	↘Subchondral bone alteration ↘Pain	[41]
IL1β injection (rat)	Morin (polyphenol: flavonoid)	Herbs	Intra-articular injection	3 h before IL1β injection	50 μM in 50 μL PBS	↘Total Mankin score ↘iNOS and COX2 expression	[46]
	Berberine (alkaloid)	Medicinal herbs	Intra-articular injection	3 h before IL1β injection	10 μM or 100 μM in 50 μL PBS	↘Total Mankin score ↘MMPs (-1, -3 and -13) expression ↗TIMP expression ↘NO and GAG release	[36]

TABLE 1 (*Continued*)

Animal model of OA	Nutraceutical	Origin	Administration route	Duration treatment	Dose	Main results	Refs
Papain injection (rat)	Sesamine (polyphenol: lignan)	Sesame	Intra-articular injection	Every 5 days for 25 days after last papain injection	1 or 10 μM	↗Cartilage thickness ↘Chondrocyte disorganization ↗PG and collagen content ↗Safranin O staining	[47]
Chymopapain-induced OA (rabbit)	Chondroitin sulfate (GAG)	Shark skeleton	Orally	Daily for 84 days	80 mg/kg (Condrosulf®)	↗GAG content	[48]
Spontaneous							
STR-Ort strain (mouse)	MSM (organic sulfur compound)	Grains, vegetables, fruit and cow's milk	Orally	13 weeks	0.06–6 g/kg/day	↘Total Mankin score ↘Articular cartilage damage ↘Meniscus injury	[49]
STR-1N strain (mouse)	Vitamins (E, C, A, B2 and B6) and selenium (vitamins and micronutrients)	Fruit, vegetables and liver	Orally	12 months	Enriched in vitamins and 2 mg/kg selenium	↘OA incidence ↘Cartilage erosion and tide mark exposure ↗Antioxidative enzyme expression	[50]
DH strain (guinea pig)	*n*-3 PUFAs (Lipid)	Oils (olive) and fish	Orally	20 weeks	5% of *n*-6:*n*-3 (1.5:1)	↘Total OARSI score ↘MMP2 and MMP9 secretion ↗GAG and collagen content ↗Subchondral bone parameters	[51]
Spontaneous OA (dog)	Chondroitin sulfate (GAG)	Quality elk velvet antler	Orally	Daily for 60 days	1 capsule of 280 mg per 20 kg body weight	↗Activity and reactivity	[42]
	n-3 PUFAs (lipid)	Flaxseed and fish	Orally	13 weeks	Diet supplemented with 1.08% EPA and DHA	↗Functional mobility	[43]
Clinical OA (cat)	*n*-3 PUFAs (lipid)	Oil (olive) and fish	Orally	10 weeks	ETA 15 mg/ml, EPA 500 mg/ml DHA 100 mg/ml, 1 ml/kg	↗Mobility and activity	[44]

[a] *Abbreviations*: ASU, avocado/soybean unsaponifiable; BCD, *Brachystemma calycinum* D don; CMC, carboxymethylated chitin; COX, cyclooxygenase; CS, chondroitin sulfate; DH, Dunkin-Hartley; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; GAG, glycosaminoglycan; GH, glucosamine hydrochloride; GSPE, grape seed procyanidin extract; HSP, heat-shock proteins; iNOS, inducible NO synthase; IU, international unit; MCL, medial cruciate ligament; MIA, mono-iodoacetate; MSM, methylsulfonylmethane; *n*-3, omega-3; *n*-6, omega 6; PG, proteoglycan; TGF, tumor growth factor; TUNEL, dUTP nick end labeling.

control of the administered dose compared with diet supplementation [26,34,43,44,49–51]. Nevertheless, oral administration must take into account both the manufacturing process and compound stability (e.g. susceptibility to oxidation and thermolability) to preserve its bioactivity.

Pharmacokinetic and bioavailability

Nutraceuticals have to be absorbed from the intestine and can be subsequently modified (e.g. deglycosylation, glucuronate modification and/or conjugation with sulfates), which can result in metabolites that are chemically distinct from the native molecules found in food [52]. Given that the fate of many nutraceuticals is unknown, several studies have investigated the effects of intra-articular injections of nutraceuticals before and/or after the induction of experimental OA (Table 1). Using this procedure, the authors examined the local and direct effects of nutraceuticals on joint cells. Although promising, results obtained using local intra-articular administration should be taken with caution because of the aforementioned reasons. It is important to keep in mind that nutraceuticals are meant to be part of the daily diet becoming digested and absorbed. The resulting metabolites that reach the peripheral circulation and the tissues are different from those nutrients present in the supplemented diet. However, this does not mean that the metabolite is inactive. For example, the metabolic fate of dietary polyphenols is well known [53] and might determine the nutrient bioactivity. This was well illustrated by Yang *et al.*, who reported that hesperitin, a metabolite of hesperidin (a polyphenol found in citrus fruits), contained in serum of hesperidin-fed rats, showed potent anti-inflammatory activity, whereas the native hesperidin molecule did not [54]. Considering the impact of metabolic modifications on nutraceutical bioactivity, the relevance of findings obtained in studies using intra-articular administration of nutraceuticals must be carefully interpreted or confirmed using diet-supplemented approaches. Surprisingly, none of the studies cited here have investigated the bioavailability of the tested compounds upon both administration routes.

In addition to the pharmacokinetic aspects, another key parameter involves the bioavailability of nutraceuticals and their metabolites in serum. Manach *et al.* [53] and Kroon *et al.* [55] reported that the maximum human plasma concentration of polyphenols or their metabolites was usually reached between 1 and 6 h following a polyphenol-rich meal ranging from 0.1 to 10 Mmol/L. Notably, within this concentration range, these compounds were shown to exhibit chondroprotective activities *in vitro* on primary articular chondrocytes [56]. Apart from these latter studies, none of the other reports presented in this review measured the plasma concentration of nutraceuticals (or of their metabolites) following diet supplementation.

Experimental design

Four distinct protocols were reported that allowed assessment of the preventive or curative potential of nutraceuticals (Fig. 2). The first model was designed to assess the preventive nature of compounds (Fig. 2a). For this, the compounds, capsaicin [41], morin and berberine [36,46], were administered to the animal before disease development. Nutraceuticals given as a preventive treatment might also be held after natural development of OA [49–51] or after surgically induced OA [27] (Fig. 2b). In the third protocol design, OA was induced in animals via surgery or chemical induction, and immediately treated with nutraceuticals [23,26,29,35,38,39,45] (Fig. 2c), thereby examining whether nutraceuticals could counteract the development of OA and its progression. Finally, the last design involved a delay in the start of treatment from 3 days to 1 month [22,24,25,28,30–32,40,47] (Fig. 2d).

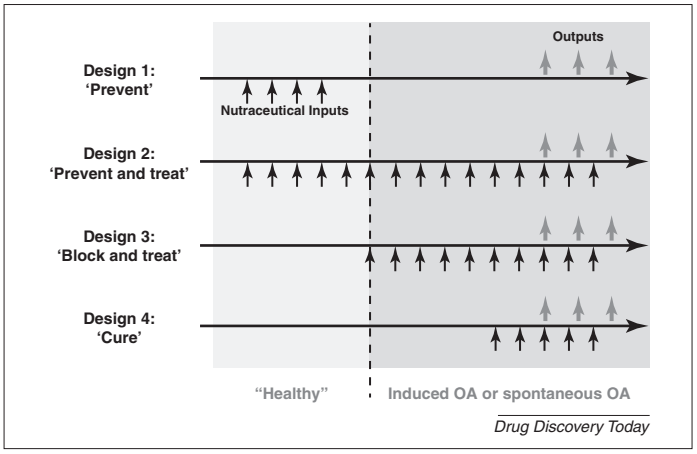

FIGURE 2

Experimental designs reported in the literature to evaluate the effectiveness of nutraceuticals on osteoarthritis (OA). To evaluate the potential to prevent or alleviate the development of symptomatic disease, nutraceuticals could be supplied before the induction of pathogenesis (Design 1). This supplementation could then be maintained to slow down the progression of OA (Design 2). Nutraceuticals can also be tested to reduce or cure the pathology (Designs 3 and 4, respectively).

Nevertheless, even though the aforementioned experimental designs collectively illustrated that nutraceuticals represent anti-OA candidates, none demonstrated a total inhibition of induced-OA and only two of these protocols could be clinically translated to humans (Fig. 2b,d).

Evaluation of nutraceutical therapy

OA grading is generally determined through histology and appears to be the gold standard for assessing nutraceutical efficiency. Mankin and OARSI scores were the most used in the studies analyzed here. The Mankin score is historically the oldest (1971) and was developed in a study that examined femoral heads removed during arthroplastic surgery in patients with advanced OA. Therefore, this method is not effective for evaluating early OA [57]. By contrast, the OARSI score (created in 1998) takes subchondral bone changes into consideration. In addition, it assesses cellular and histological cartilage damage. Considering this information, it is surprising that the most popular scoring system used in the studies referenced in Table 1 was the Mankin method. Indeed, the OARSI standardized scoring system for cartilage and periarticular tissue health allows a priori comparison of the effects of nutraceuticals, despite the lack of homogeneity in treatments, models and standard controls between independent studies. To understand thoroughly the mechanisms underlying the actions of nutraceuticals, gene expression analyses are commonly used. Indeed, examining the expression of genes related to inflammation [27] and cartilage anabolism [26] or catabolism [31,36] in cartilage tissue [27,31] revealed that nutraceuticals modulated these expression patterns to improve cartilage health.

Mechanistic action of nutraceuticals on OA

Anti-OA properties of nutraceutical families

Polyphenols, a nutraceutical family found in fruits, vegetables and plants, are well recognized for their anti-inflammatory and anti-oxidative activities. For this reason, they have been tested in different animal models of OA. Interestingly, polyphenols induce a reduction of oxidative stress markers [25,37,40] and inflammation [24,40] that is associated with a potent reduction in pain and structural damage [38,39]. Specifically, certain polyphenols, such as procyanidin B, chlorogenic acid, resveratrol or sesamin, can reduce chondrocyte damages (cell death and disorganization). Thus, these treatments result in an improved histological OA scores. In addition, other nutraceuticals have displayed similar results and can be used to manage OA (e.g. alkaloids [31,36,41], carbohydrate [33], organic sulfur compound [49] or certain plant extracts [35]).

Some authors fed animals with protein- [29,30] or glucosamine-supplemented [42,48] diets to counteract the loss of cartilage observed in OA. Indeed, proteins can ensure an adequate supply of substrate to stimulate cartilage anabolism. As expected, the authors observed an increase in proteoglycan content in the protein- or glucosamine-supplemented groups, resulting in the thickening of cartilage and improved histological scores compared to controls. As a consequence, improved mobility was also reported.

Poly-unsaturated fatty acids (PUFAs), which are lipids well known for their anti-inflammatory properties, have also been used to treat OA symptoms and progression [58]. In this regard, Knott *et al.* investigated the benefit of omega-3 (*n*-3) enriched diets in Dunkin-Hartley guinea pigs [51] and found that *n*-3 reduced disease parameters in OA-prone animals compared with an OA-resistant strain. Also, using clinical animals such as cats and dogs with naturally occurring OA, it was reported that *n*-3 supplemented diets could improve mobility and activity [43,44].

An important goal is to prevent the development of OA altogether. Notably, one report has indicated that nutraceuticals, especially vitamins and minerals, can reduce OA incidence in genetically modified mice (STR/1N) [50]. This OA prevention probably results from the anti-inflammatory properties of these vitamins [27].

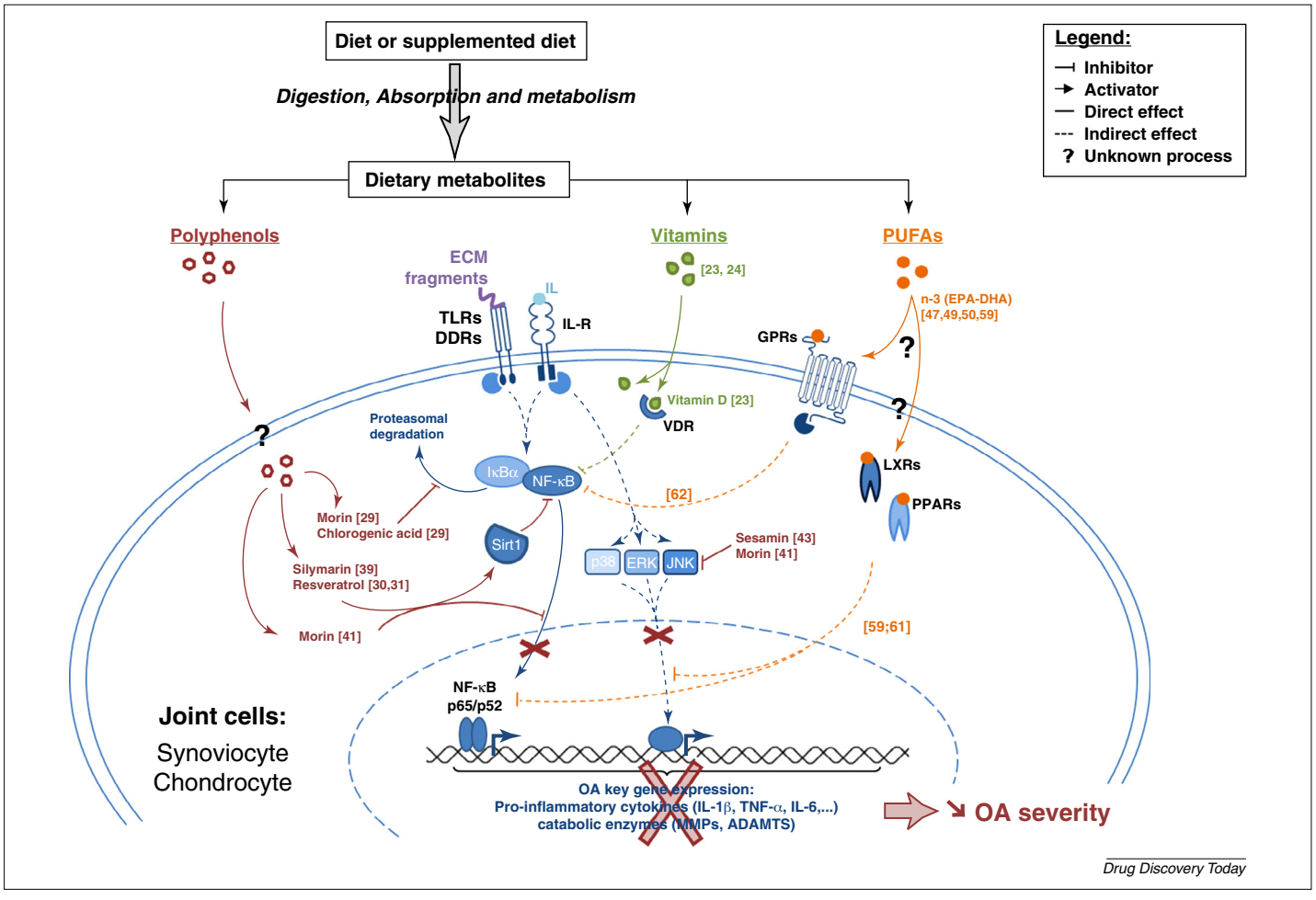

FIGURE 3

Molecular mechanisms of action of nutraceuticals on joint cells. Nutraceuticals alleviate inflammation and cartilage degradation by modulating nuclear factor (NF)-κB and mitogen-activated protein kinases [extracellular signal-regulated kinases (ERK) 1/2, p38 kinase and c-Jun N-terminal kinase (JNK)]. These critical pathways were activated by inflammatory stimuli [interleukin (IL)-1β] or extracellular matrix fragments, which are recognized as damage-associated molecular patterns (DAMPs), leading to the expression of matrix metalloproteinases (MMPs), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) and inflammatory cytokines that participate in cartilage degradation. The supplementation of nutraceuticals can block many steps of these signaling pathways to decrease osteoarthritis (OA) severity. For additional definitions, please see the main text.

Molecular mechanisms of action

The dietary supplements and intra-articular injections outlined in this review were shown to be beneficial for OA treatment, suggesting that nutraceuticals act on joint cells (synoviocytes and chondrocytes) to modulate the expression of key genes regulating OA onset. In this regard, a tempting scheme of the underlying cellular and molecular mechanisms that mediate the action of nutraceuticals is outlined in Fig. 3. Many reported nutraceutical pathways are known to inhibit nuclear factor kappa B (NF-κB) activation. NF-κB, is a key transcription factor that drives the expression of OA inducer genes in response to the activation of toll-like receptors (TLRs) and interleukin receptor (IL-R) by extracellular matrix fragments and IL1-β, respectively [59]. In addition to NF-κB activation, the expression of pro-inflammatory cytokines and catabolic enzymes can be activated by the mitogen-activated protein kinase (MAPK) pathway through the extracellular signal-regulated kinases (ERK) 1/2, p38 kinase and c-Jun N-terminal kinase (JNK). Indeed, some polyphenols (e.g. sesamin and morin) have been found to inhibit these pathways [46,47]. Among others possible modes of action, fatty acids might act directly on G protein-coupled receptors (GPRs) or other lipid-activated nuclear receptor families, such as liver X receptors (LXRs) and peroxisome proliferator activated receptors (PPARs) [60]. The activation of GPRs by natural or synthetic agonists is known to inhibit the NF-κB pathway [61]. In addition, signaling through PPARγ has been shown to interfere with NF-κB and to regulate gene expression directly to decrease inflammatory markers. Similarities have been reported from *in vitro* studies examining chondroïtin sulfate, which inhibits NF-κB nuclear translocation and phosphorylation of p38 MAPK, ERK1/2, and JNK [62]. PUFAs, similar to many other nutrients, can modulate mammalian target of rapamycin (mTOR) signaling, leading to a protective activation of autophagy in chondrocytes [58]. In addition, a nutrient-sensitive pathway dependent on sirtuin 1 (SIRT1), a class III histone deacetylase that is a member of the sirtuin family, was recently described. Notably, downregulation of SIRT1 was reported to heighten OA progression in induced models and during aging [63]. For example, SIRT1 activation in the presence of resveratrol interferes with NF-κB signaling, resulting in anti-inflammatory effects during OA [64]. Unexpectedly, none of the studies cited in this review assessed whether the nutraceuticals can activate SIRT1 *in vivo*. Overall, it appears that nutraceuticals can target different molecular pathways involved in OA progression, suggesting that they exhibit cumulative effects or act in synergy to counteract OA. Thus, these findings suggest that it would be interesting to associate several classes of nutraceutical to obtain a more efficient therapeutic strategy.

Concluding remarks and future directions

Here, we have examined several studies that highlight the effectiveness of nutraceuticals in OA management. We have also discussed various animal models of OA, which represent relevant preclinical tools for studying the effects of dietary supplements on OA. However, in regard to future clinical therapy, spontaneous OA animal models appear to recapitulate most closely the human pathology, enabling us to follow the evolution of the disease. Moreover, we have highlighted the wide range of biological effects that can be elicited by nutraceuticals, including anti-inflammatory actions, pain reduction and gain of mobility. Furthermore, a synergic therapy involving the use of different nutraceutical families might prove to be a promising approach for treating OA, because it could combine the various biological effects of these agents. Finally, encouraging results have been obtained in animals using double-blind experiments, perhaps representing a final step before initiating clinical trials to examine the use of nutraceutical therapy in patients with OA.

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References

1 Nuesch, E. *et al.* (2011) All cause and disease specific mortality in patients with knee or hip osteoarthritis: population based cohort study. *Br. Med. J.* 342, d1165
2 Leardini, G. *et al.* (2004) Direct and indirect costs of osteoarthritis of the knee. *Clin. Exp. Rheumatol.* 22, 699–706
3 Loeser, R.F. *et al.* (2012) Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum.* 64, 1697–1707
4 Goldring, M.B. and Marcu, K.B. (2009) Cartilage homeostasis in health and rheumatic diseases. *Arthritis Res. Ther.* 11, 224
5 Amin, A.R. *et al.* (2000) COX-2, NO, and cartilage damage and repair. *Curr. Rheumatol. Rep.* 2, 447–453
6 Hart, H.F. *et al.* (2012) Quadriceps volumes are reduced in people with patellofemoral joint osteoarthritis. *Osteoarthritis Cartilage* 20, 863–868
7 Chappard, C. *et al.* (2006) Subchondral bone micro-architectural alterations in osteoarthritis: a synchrotron micro-computed tomography study. *Osteoarthritis Cartilage* 14, 215–223
8 Pesesse, L. *et al.* (2011) Osteochondral plate angiogenesis: a new treatment target in osteoarthritis. *Joint Bone Spine* 78, 144–149
9 Mapp, P.I. *et al.* (2008) Angiogenesis in two animal models of osteoarthritis. *Osteoarthritis Cartilage* 16, 61–69
10 Nakamura, H. (2011) Application of glucosamine on human disease: osteoarthritis. *Carbohydr. Polym.* 84, 835–839
11 Clouet, J. *et al.* (2009) From osteoarthritis treatments to future regenerative therapies for cartilage. *Drug Discov. Today* 14, 913–925
12 Felson, D.T. *et al.* (2000) Osteoarthritis: new insights. Part 2: treatment approaches. *Ann. Intern. Med.* 33, 726–737
13 Akhtar, N. and Haqqi, T.M. (2012) Current nutraceuticals in the management of osteoarthritis: a review. *Ther. Adv. Musculoskelet. Dis.* 4, 181–207
14 Zeisel, S.H. (1999) Regulation of 'nutraceuticals'. *Science* 285, 1853–1855
15 Kalra, E.K. (2003) Nutraceutical: definition and introduction. *AAPS PharmSci.* 5, E25
16 Das, L. *et al.* (2012) Role of nutraceuticals in human health. *J. Food Sci. Technol.* 49, 173–183
17 Henrotin, Y. *et al.* (2011) Nutraceuticals: do they represent a new era in the management of osteoarthritis? A narrative review from the lessons taken with five products. *Osteoarthritis Cartilage* 19, 1–21
18 McAlindon, T.E. *et al.* (1996) Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. *Ann. Intern. Med.* 125, 353–359
19 Vangsness, C.T., Jr *et al.* (2009) A review of evidence-based medicine for glucosamine and chondroitin sulfate use in knee osteoarthritis. *Arthroscopy* 25, 86–94
20 Gregory, M.H. *et al.* (2012) A review of translational animal models for knee osteoarthritis. *Arthritis* 2012, Article ID 764621, 14 pages, http://dx.doi.org/10.1155/2012/764621
21 Little, C.B. and Smith, M.M. (2008) Animal models of osteoarthritis. *Curr. Rheumatol. Rev.* 4 (3)
22 Aini, H. *et al.* (2012) Procyanidin B3 prevents articular cartilage degeneration and heterotopic cartilage formation in a mouse surgical osteoarthritis model. *PLoS One* 7, e37728
23 Chen, W.P. *et al.* (2011) Anti-arthritic effects of chlorogenic acid in interleukin-1beta-induced rabbit chondrocytes and a rabbit osteoarthritis model. *Int. Immunopharmacol.* 11, 23–28

24 Elmali, N. *et al.* (2005) Effect of resveratrol in experimental osteoarthritis in rabbits. *Inflamm. Res.* 54, 158–162
25 Wang, J. *et al.* (2012) Effect of resveratrol on cartilage protection and apoptosis inhibition in experimental osteoarthritis of rabbit. *Rheumatol. Int.* 32, 1541–1548
26 Ham, K.D. *et al.* (2004) Effects of long-term estrogen replacement therapy on articular cartilage IGFBP-2, IGFBP-3, collagen and proteoglycan levels in ovariectomized cynomolgus monkeys. *Osteoarthritis Cartilage* 12, 160–168
27 Castillo, E.C. *et al.* (2012) Effects of Vitamin D supplementation during the induction and progression of osteoarthritis in a rat model. *Evid. Based Complement. Alternat. Med.* 156563
28 Kobayashi, T. *et al.* (2005) Fursultiamine, a vitamin B1 derivative, enhances chondroprotective effects of glucosamine hydrochloride and chondroitin sulfate in rabbit experimental osteoarthritis. *Inflamm. Res.* 54, 249–255
29 Boileau, C. *et al.* (2009) Protective effects of total fraction of avocado/soybean unsaponifiables on the structural changes in experimental dog osteoarthritis: inhibition of nitric oxide synthase and matrix metalloproteinase-13. *Arthritis Res. Ther.* 11, R41
30 Cake, M.A. *et al.* (2000) Modification of articular cartilage and subchondral bone pathology in an ovine meniscectomy model of osteoarthritis by avocado and soya unsaponifiables (ASU). *Osteoarthritis Cartilage* 8, 404–411
31 Zhou, X. *et al.* (2013) Tetrandrine inhibits the Wnt/β-catenin signalling pathway and alleviates osteoarthritis: an *in vitro* and *in vivo* study. *Evid. Based Complement. Alternat. Med.* 809579
32 Tiraloche, G. *et al.* (2005) Effect of oral glucosamine on cartilage degradation in a rabbit model of osteoarthritis. *Arthritis Rheum.* 52, 1118–1128
33 Hongbin, W. *et al.* (2004) Carboxymethylated chitin reduces MMP-1 expression in rabbit ACLT osteoarthritic cartilage. *Ann. Rheum. Dis.* 63, 369–372
34 Lippiello, L. *et al.* (2000) In vivo chondroprotection and metabolic synergy of glucosamine and chondroitin sulfate. *Clin. Orthop. Relat. Res.* 381, 229–240
35 Boileau, C. *et al.* (2009) Oral treatment with a *Brachystemma calycinum* D don plant extract reduces disease symptoms and the development of cartilage lesions in experimental dog osteoarthritis: inhibition of protease-activated receptor 2. *Ann. Rheum. Dis.* 69, 1179–1184
36 Hu, P.F. *et al.* (2011) Protective effects of berberine in an experimental rat osteoarthritis model. *Phytother. Res.* 25, 878–885
37 Chen, W.P. *et al.* (2012) Morin inhibits interleukin-1beta-induced nitric oxide and prostaglandin E2 production in human chondrocytes. *Int. Immunopharmacol.* 12, 447–452
38 Hadipour-Jahromy, M. and Mozaffari-Kermani, R. (2010) Chondroprotective effects of pomegranate juice on monoiodoacetate-induced osteoarthritis of the knee joint of mice. *Phytother. Res.* 24, 182–185
39 Woo, Y.J. *et al.* (2011) Grape seed proanthocyanidin extract ameliorates monosodium iodoacetate-induced osteoarthritis. *Exp. Mol. Med.* 43, 561–570
40 Ashkavand, Z. *et al.* (2012) Silymarin potentiates the anti-inflammatory effects of Celecoxib on chemically induced osteoarthritis in rats. *Phytomedicine* 19, 1200–1205
41 Kalff, K.M. *et al.* (2010) Pre-treatment with capsaicin in a rat osteoarthritis model reduces the symptoms of pain and bone damage induced by monosodium iodoacetate. *Eur. J. Pharmacol.* 641, 108–113
42 Moreau, M. *et al.* (2004) Clinical evaluation of a powder of quality elk velvet antler for the treatment of osteoarthrosis in dogs. *Can. Vet. J.* 45, 133–139
43 Moreau, M. *et al.* (2004) Effects of feeding a high omega-3 fatty acids diet in dogs with naturally occurring osteoarthritis. *J. Anim. Physiol. Anim. Nutr.* 97, 830–837
44 Corbee, R.J. *et al.* (2012) The effect of dietary long-chain omega-3 fatty acid supplementation on owner's perception of behaviour and locomotion in cats with naturally occurring osteoarthritis. *J. Anim. Physiol. Anim. Nutr.* 97, 846–853
45 Fujita, S. *et al.* (2011) Combined microwave irradiation and intraarticular glutamine administration-induced HSP70 expression therapy prevents cartilage degradation in a rat osteoarthritis model. *J. Orthop. Res.* 30, 401–407
46 Chen, W.P. *et al.* (2012) Morin exerts antiosteoarthritic properties: an *in vitro* and *in vivo* study. *Exp. Biol. Med.* 237, 380–386
47 Phitak, T. *et al.* (2012) Chondroprotective and anti-inflammatory effects of sesamin. *Phytochemistry* 80, 77–88
48 Uebelhart, D. *et al.* (1998) Protective effect of exogenous chondroitin 4,6-sulfate in the acute degradation of articular cartilage in the rabbit. *Osteoarthritis Cartilage* 6 (Suppl. A), 6–13
49 Ezaki, J. *et al.* (2013) Assessment of safety and efficacy of methylsulfonylmethane on bone and knee joints in osteoarthritis animal model. *J. Bone Miner. Metab.* 31, 16–25
50 Kurz, B. *et al.* (2002) Dietary vitamins and selenium diminish the development of mechanically induced osteoarthritis and increase the expression of antioxidative enzymes in the knee joint of STR/1N mice. *Osteoarthritis Cartilage* 10, 119–126
51 Knott, L. *et al.* (2011) Regulation of osteoarthritis by omega-3 (n-3) polyunsaturated fatty acids in a naturally occurring model of disease. *Osteoarthritis Cartilage* 19, 1150–1157
52 Rein, M.J. *et al.* (2013) Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. *Br. J. Clin. Pharmacol.* 75, 588–602
53 Manach, C. *et al.* (2004) Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 79, 727–747
54 Yang, H.L. *et al.* (2011) Antioxidant and anti-inflammatory potential of hesperetin metabolites obtained from hesperetin-administered rat serum: an ex vivo approach. *J. Agric. Food Chem.* 60, 522–532
55 Kroon, P.A. *et al.* (2004) How should we assess the effects of exposure to dietary polyphenols *in vitro*? *Am. J. Clin. Nutr.* 80, 15–21
56 Shen, C.L. *et al.* (2012) Dietary polyphenols and mechanisms of osteoarthritis. *J. Nutr. Biochem.* 23, 1367–1377
57 Pritzker, K.P. *et al.* (2006) Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthritis Cartilage* 14, 13–29
58 Villalvilla, A. *et al.* (2013) Lipid transport and metabolism in healthy and osteoarthritic cartilage. *Int. J. Mol. Sci.* 14, 20793–20808
59 Scanzello, C.R. *et al.* (2008) Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? *Curr Opin. Rheumatol.* 20, 565–572
60 Calder, P.C. (2013) Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br. J. Clin. Pharmacol.* 75, 645–662
61 Wauquier, F. *et al.* (2013) The free fatty acid receptor G protein-coupled receptor 40 (GPR40) protects from bone loss through inhibition of osteoclast differentiation. *J. Biol. Chem.* 288, 6542–6551
62 Jomphe, C. *et al.* (2008) Chondroitin sulfate inhibits the nuclear translocation of nuclear factor-kappaB in interleukin-1beta-stimulated chondrocytes. *Basic Clin. Pharmacol. Toxicol.* 102, 59–65
63 Matsuzaki, T. *et al.* (2013) Disruption of Sirt1 in chondrocytes causes accelerated progression of osteoarthritis under mechanical stress and during ageing in mice. *Ann. Rheum. Dis.* 73, 1397–1404
64 Hubbard, B.P. *et al.* (2013) Evidence for a common mechanism of SIRT1 regulation by allosteric activators. *Science* 339, 1216–1219