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FRONTIER

Glucosamine and chondroitin for the treatment of osteoarthritis

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Abstract

The prevalence of primary or idiopathic osteoarthritis (OA) of knee and hip joints has substantially increased in general population during the last decades. Analgesics and non-steroidal anti-inflammatory drugs are currently extensively used as non-surgical treatment options. However, they act as symptomatic treatments, not offering a cure of OA and they are accused for an increased risk of adverse events. Glucosamine (GL) and chondroitin (CH) are nutritional supplements that have recently gained widespread use as treatment options for OA. They potentially or theoretically act as chondroprotectors or/and as "disease-modifying OA drugs" offering not only symptomatic relief but also alteration of the natural history of OA. However, although many studies have showed a significant treatment effect, accompanied with remarkable safety, there is still controversy regarding their relative effectiveness compared with placebo or other treatments. The scope of this review is to present and critically evaluate the current evidence-based information regarding the administration of GL and CH for the treatment of knee or hip OA. Our focus is to investigate the clinical efficacy and safety after the use of these supplements. An effect of GL and CH on both clinical and radiological findings has been shown. However, only a few high-quality level I trials exist in the literature, especially on the assessment of radiological progression of OA. The effect sizes are generally small and probably not clinically relevant. Even the validity of these results is limited by the high risk of bias introduced in the studies. Both GL and CH seem to be safe with no serious adverse events reported. There is currently no convincing information for the efficacy of GL and CH on OA.

Key words: Glucosamine; Chondroitin; Osteoarthritis; Knee; Cartilage

Core tip: In this review we present and critically evaluate the current information regarding the administration of glucosamine (GL) and chondroitin (CH) for the treatment of knee or hip osteoarthritis. A clinical and radiological effect of GL and CH has been shown. However, only a few high quality trials exist. The effect

sizes are small and probably not clinically relevant. The validity of these results is limited by high risk of bias introduced in the studies. Both GL and CH seem to be safe with no serious adverse events but there is currently no convincing information for their efficacy as treatment options in osteoarthritis.

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INTRODUCTION

The prevalence of primary or idiopathic osteoarthritis (OA) of knee and hip joints has substantially increased in general population during the last decades. The aging of the population and the increment of life expectance are contributing factors; however, there is also a high incidence of OA in younger ages[1,2]. Approximately 5% of the population aged between 35 and 54 years has radiographic signs of knee osteoarthritis, which reaches 30% for ages between 45 and 65[3]. Except from post-traumatic OA, a reason for younger patients may be the wide participation in high competitive sports and the increment of recreational athletes even in not regularly and inadequately trained population. This subjects their joints to distracting repetitive forces that may lead to progressive cartilage damage and subsequently to secondary or posttraumatic OA.

Focal cartilage lesions usually occur at a first stage, often remaining asymptomatic. Untreated or under-treated lesions may lead to OA. The treatment of OA in elder patients is well clarified and accepted to be joint reconstruction *via* an arthroplasty (either hip or knee). However, arthroplasty may be considered a salvage procedure requiring a modification of daily life postoperatively, not participation in contact sports or high impact sports and is subject to revision surgery after a certain period of time. Therefore this treatment option does not apply to the more active and/or younger patients, even those with severe OA.

Therefore, there is increasing need for treating OA with less invasive interventions, with pharmaceutical agents being the most favourite especially for younger age groups. Analgesics and non-steroidal anti-inflammatory drugs are currently extensively used[4-6]. However, they frequently cause serious adverse events, including the gastrointestinal or cardiovascular system. Given also that they rather act as symptomatic treatment, not offering a cure of OA, a long-term use is usually required, increasing the risk of such events[4,5].

An ideal treatment would not only reduce the symptoms but additionally modify the natural history of OA, slowing or even altering the inflammation and destructive effect on the articular cartilage and joint tissues. Such substances that protect the articular cartilage during the course of OA have been termed as "chondroprotective agents" or "chondroprotectors". When these agents appear to alter the course of the disease (*e.g*., by modifying the biochemical cascades that contribute to the OA), they are termed "disease-modifying OA drugs" (DMOADs). Such agents aim to protect the joint cartilage along with the subchondral bone and synovial membrane, which are the main structures of the joint[7-9].

Glucosamine (GL) and chondroitin (CH) are nutritional supplements that have gained widespread use. They are two main categories of agents potentially or theoretically acting as chondroprotectors and/or as DMOADs. Although many studies have been published showing a significant treatment effect, accompanied with remarkable safety, there is still controversy regarding their relative effectiveness compared with placebo or other treatments, their cost-effectiveness and the need for insurance coverage of the therapy cost[10-12]. Due to methodological and bias concerns, these studies have failed to persuade most of the big national insurance committees (like FDA or NICE) or the biggest scientific societies (like EULAR, ACG EULAR or OARSI) to include GL and CH as first line treatment options in their guidelines[13-19]. However despite this, global sales of GL supplements reached almost $2bn in 2008 in United States, after an increase of about 60% compared with 2003, with a forecasted continued growth that would reach $2.3bn in 2013[20].

The scope of this review is to present and critically evaluate the current evidence-based information regarding the administration of GL and CH for the treatment of knee or hip OA. Our focus is to investigate the clinical efficacy and safety after the use of these supplements. Initially we will present the theoretical mechanism of action of these agents, through which they may affect the progress of OA. Next, we will present the clinical evidence, mainly based on the level Ⅰ information from systematic reviews (SRs) of randomised control trials (RCTs). Finally, we will discuss the information along with probable factors that may contribute to a safe conclusion regarding the efficacy and safety of the use of GL and CH for the treatment of osteoarthritis.

BACKGROUND

Molecular structure of articular cartilage and mechanism of primary OA

Articular cartilage has a vast preponderance of extracellular matrix (composed of collagen and proteoglycans), in which cells (chondrocytes) are distributed sparsely. Collagen fibrils (mainly of type Ⅱ collagen) form the framework of articular cartilage[21]. The proteoglycan aggregate is an aggregation of proteoglycan monomers attaching to the filamentous hyaluronan backbone and fills the space of the collagen network[22]. The proteoglycan molecules (also called aggrecans) consist of numerous long-chain glycosaminoglycans (GAGs)

linked to a core protein. Such GAGs (CH sulfate and keratan sulphate) are linear polymers composed of sugar residues[23]. They are composed of repeating units of N-acetylgalactosamine and glucuronic acid (in CH sulphate) and N-acetylglucosamine and galactose (in keratan sulphate). GAGs are negatively charged, so they attract a large quantity of water molecules. More than 70% of the net weight of cartilage consists of water. Synovial fluid produced from synovial cells, lubricates the joint surfaces and also provides cartilage with oxygen and nutrition.

In OA, matrix metalloproteinases (MMPs) and aggrecanases produced by inflamed synovial cells and the diseased chondrocytes result in a gradual degradation of collagen and proteoglycan molecules. Lytic enzymes released as a result of this degradation also enhance synovial inflammation and induce chondrocytes' apoptosis. The inflammation leads to progressive cartilage degradation. The network described above is gradually destructed. Loss of aggrecans from the extracellular matrix leads to a change in the biomechanical properties of the cartilage tissue. This adds to an increased mechanical wear and would result in an accelerated damage of articular cartilage and eventually to OA. This mechanism of OA may be triggered by traumatic lesions and degradation of focal lesions of cartilage, chondrocyte apoptosis and consequent release of lytic enzymes entering the above described cascade of events.

Prostaglandins released by synoviocytes and chondrocytes during this inflammatory cyclic reaction of cartilage degradation are also known to enhance pain and inflammation.

The above suggested mechanism is primarily apparent in primary osteoarthritis, which is characterised by a generalized cellular dysfunction starting with focal degradation in the most loaded areas of the joint articular surface. In secondary cases of osteoarthritis, other factors also contribute to the joint damage. For example in posttraumatic OA a traumatic focal cartilage lesion may trigger this cascade of degradation. In this case the combination of the mechanic break down in the lesion area and the enzymatic degradation of the damaged cartilage finally lead to OA.

In vitro and animal studies

GL: GL is a water-soluble amino monosaccharide and one of the most abundant monosaccharides in the human body. It is present in high quantities in articular cartilage, being a normal constituent of GAGs in cartilage matrix and also in the synovial fluid. It is a constituent of keratan sulphate. There are two forms: Glucosamine sulphate (GS) and glucosamine hydrochloride (GH).

The way that exogenous administration of GL may work in OA is not yet fully defined. It is believed that GL may have an important role in regulating the anabolic processes of cartilage and also in the synthesis of synovial fluid. Additionally it may inhibit the degenerative and catabolic process of OA with its anti-inflammatory and even antioxidant properties.

It is reported that GL may affect the cytokine-mediated pathways regulating inflammation, cartilage degradation, and immune responses[24,25]. It appears to have immune-modulatory activity inhibiting the expression and/or activity of catabolic enzymes such as phospholipase A2, MMPs or aggrecanases[24-27]. GL reduces or regulates interleukin-1 (IL-1) levels in synovial fluid and inhibits the actions of catabolic enzymes in the joint[28-30]. This reduces inflammation and cartilage degradation potentially altering the progression of OA. Except from its anti-catabolic action, it has been suggested that GL sulphate has an anabolic effect by stimulating cultured human chondrocytes to synthesize proteoglycans and has been reported to be a substrate for new CH sulphate synthesis[24,31]. It has also found to inhibit gene expression of OA cartilage *in vitro*[31]. Finally, GL may act by inducing the production of hyaluronic acid by the synovial membrane[31]. Along with its indirect effect on the cartilage metabolism, being a precursor of GAGs, it is also possible that supplementation with GL may help promote GAG synthesis or reduce its degradation.

Animal studies have also supported the anabolic and/or anti-catabolic effect of GL on cartilage. A GL analogue has demonstrated both anti-arthritic and anti-inflammatory properties in rats[32]. Another study reports a positive effect on cartilage, enhancing the rate of new proteoglycan synthesis[33] and others have confirmed the effectiveness of GL in delaying the cartilage degradation and the progression and severity of OA[34]. Long-term oral administration of GL sulphate also reduced the destruction of cartilage and upregulation of MMP-3 mRNA in a model of spontaneous osteoarthritis in Harley guinea pigs[30,35]. However, the preparation used in many of *in vitro* and *in vivo* studies was not a GL sulphate ester but a preparation in which GL and sulphate occurred as two single molecules in crystalline form[36].

CH: CH sulfate is a sulfated GAG being also a major component of the extracellular matrix of articular cartilage. It is found attached to proteins as part of the aggrecan of the cartilage. It plays a major role in creating considerable osmotic pressure that expands the matrix and places the collagen network under tension[37]. It provides cartilage with resistance and elasticity allowing it to resist tensile stresses during various loading conditions[38].

Similarly to GS, the exogenous administration of chondroitin sulphate (CS) has been suggested to act against OA by three main mechanisms; anabolic effect by stimulating the production of extracellular matrix of cartilage, suppression of inflammatory mediators and inhibition of cartilage degeneration[21]. Studies have demonstrated that CS counteracts the action of IL-1b (a factor that induces articular inflammation and

cartilage degeneration), thus playing a chondroprotective role[39,40]. Additionally an effect on subchondral bone had been suggested by reducing the resorptive activity in subchondral bone[41,42].

Proteoglycan content in cartilage was also significantly higher in animals treated with oral or intramuscular administration of CS than that in control animals[43]. It has been shown that CS significantly decreases collagenolytic activity[44]. Other studies suggested that the benefits of CS on degenerative osteoarthritic chondrocytes are larger than those on normal chondrocytes[39,45].

Bioavailability

As described above, both GL and CH are components of the extracellular matrix of articular cartilage. Experimental studies have also suggested an additional action in inflammatory pathways that contribute to OA. Provided this, their external administration has been widely considered as a treatment option for OA.

GL and CH have been used for medicinal purposes for nearly 40 years[46]. However, their bioavailability after oral administration in humans is a subject still under debate. A key issue would be the absorption of these agents through their passing from the gastrointestinal system.

In mammals, the major site of their metabolism and degradation is the liver, but the exact mechanism is unclear[21]. Published information is rather controversial. Early pharmacodynamic studies inferred absorption only indirectly. Laboratory work has suggested that GL is substantially degraded in the gastrointestinal tract[47]. Other studies show that despite its large molecular size, ingested CH is partially absorbed in the intestine and some of it may reach joints[10,48]. A pharmacokinetic study in dogs, showed that GL (hydrochloride) is absorbed with a bioavailability of about 10%-12% from single or multiple doses[49]. In humans, serum GL levels following an oral dose of 1.5 g GL sulfate do not appear to exceed 12 mmol/L. Animal studies have also shown that after oral administration of GL hydrochloride, synovial GL concentrations are higher in joints with synovial inflammation compared to levels attained in healthy joints[50].

Regarding CS, different bioavailability and pharmakokinetic variables have been reported, usually depending on the study methodology or the CS characteristics[51]. A bioavailability of 10%-20% has been reported in earlier studies[52-54]. Study in humans has shown a significant increase in plasma levels (more than 200% compared with pre-dose levels) over a 24-h period[48]. Use of labelled CS has shown a high level of CS, observed in the human synovial fluid and articular cartilage after oral administration[53]. A limitation to the studies provided above is that both GL and CS are drugs of biological origin. Thus, their measurement in biological fluids does not discriminate the drug from endogenous molecules.

CLINICAL EVIDENCE

Based on laboratory and animal studies, it has been suggested that GL and CH may be effective on preserving cartilage in early OA, and hence might slow down its progression. This would result in a relief from symptoms including pain and stiffness. This claim was also based on clinical studies that reported a clinical benefit after oral administration. However, recent SRs have cast doubt on this.

Quite early, in 2000, a large SR of RCTs assessed the efficacy and safety of GL (GS or GH) and CH[55]. Assessing 15 RCTs, the authors found moderate effect sizes for GL (0.44, 95%CI: 0.24-0.64) and large effects for CH (0.96, 95%CI: 0.63-1.3). They also extensively investigated the quality of information provided by these studies. A high risk of bias was reported, with poor methodology and poor reporting among the included trials. In all but two trials there was some level of manufacturer sponsorship, while none of the studies reported independent funding from a governmental or non-for-profit organization. They also found that pooled effect sizes were substantially higher compared to those of lower quality or smaller trials, which seem to exaggerate the efficacy of both GL and CH. A high risk of publication bias was also shown on funnel plots, suggesting a high probability of not reporting of small trials or of those with small or null treatment effect.

Richy *et al*[56] assessed also 15 RCTs, concluding to a superiority of GL and CH in clinical and radiological findings. Although the authors assessed the quality of the included trials, no further analysis was performed to detect any association with the effect sizes.

Wandel *et al*[11] assessed RCTs that compared CS, GS, GH, or the combination of any two with placebo or head to head. Small trials and ones using subtherapeutic doses were excluded. A network meta-analysis of 10 trials was conducted. In 5 trials, GS was compared with placebo, in 3 CS with placebo, and one compared GH, CS and their combination with placebo. In another placebo controlled trial GS was used; however, after 80% of the patients had been treated, the investigators were forced to change into GH because the manufacturer of GS declined to supply matching placebos[57]. Seven of the trials were funded by manufacturers. Joint pain was extracted in nine time-windows starting from "up to 3 mo", up to "22 mo or more". Effect sizes for joint pain were -0.17 (95%CI: -0.28 to -0.05) for GL, -0.13 (95%CI: -0.27 to 0.00) for CH, and -0.19 (95%CI: -0.37 to 0.00) for the combination suggesting a close to null effectiveness of the interventions. Stratified analysis revealed that the estimated differences between supplements and placebo were significantly more pronounced in industry funded trials [by on average, 0.5 cm (0.1 to 0.9 cm) in a 10-cm VAS scale, $P = 0.02$]. The analysis of 6 trials providing outcome on radiological joint space, showed no clinically relevant effect on joint space narrowing for

any of the interventions. No differences were found in adverse events, and withdrawals or drop-outs because of adverse events. The authors concluded that CH, GL, and their combination do not have a clinically relevant effect on perceived joint pain or on joint space narrowing. They suggested that health authorities and health insurers should not cover the costs of these preparations, and new prescriptions to patients who have not received other treatments should be discouraged.

Vlad *et al*[58] analysed 15 RCTs comparing GL (12 GS and 3 GH) with placebo. Industry funding was reported for 11 trials, while 13 studies used an industry-supplied drug. Rottapharm provided GS in 8 trials and contributed to a ninth trial. The authors reported a marked heterogeneity among trials. They found marked differences between subgroups of trials when grouped by various trial characteristics. Overall, they found a pooled effect size of 0.35 (95%CI: 0.56 to 0.14) in favour of GL. However, there was substantial heterogeneity among trials, questioning the reliability of this finding. This heterogeneity remained high in the industry-funded trials but not in the independent trials. The 11 industry-funded trials had a pooled effect size of 0.47 (95%CI: 0.24-0.70) favouring GL; however a null effect size was found when only the 4 non-industry-funded trials were analysed 0.05 (95%CI: -0.32 to 0.41). Trials with Rottapharm products (a GS product) showed an increased effect size compared with trials with other products (P = 0.01.) In general, heterogeneity was absent and effect sizes were smaller in high quality, more recently published and not funded trials, suggesting a high risk of bias for the overall quality of provided information in the related literature. Trials using GS had an effect size favouring the intervention (0.44, 95%CI: 0.18 to 0.70) although GH did not show superiority over placebo. High heterogeneity was found in both cases. The authors concluded that there is sufficient information to conclude that GH lacks efficacy for pain in OA. Among GS trials, marked heterogeneity existed; therefore no definitive conclusion about efficacy is possible.

Reichenbach *et al*[10] assessed 22 RCTs or quasi-RCT trials that compared CH with placebo or no intervention. The authors also reported a low quality of evidence as only a few trials had an adequate generation of allocation sequence (1 study) or adequate concealment (2 studies) or followed an intention to treat analysis (3 studies). The meta-analysis of 20 trials providing pain outcomes suggested a pooled large effect size that favours CH sulphate -0.75 (-0.99 to -0.50), corresponding to a difference of 1.6 cm on a 10 cm VAS. However, the heterogeneity was large (I^2 = 92%) and the funnel plot was asymmetrical suggesting high publication bias. More recent trials tended to be larger and of higher quality and included patients with lower-grade of osteoarthritis than did earlier trials. Stratified analysis found that when the analysis was restricted to methodologically sound trials of adequate sample size, there was a null effect size with low heterogeneity. From 5 trials assessing the difference of mean joint space width, the authors found a mean effect size of 0.18 SD units favouring CH, an effect size that was not clearly clinically significant. The authors finally discouraged the use of CH. In this trial only one time point was assessed per trial, which was criticised.

Another SR assessed the short-term efficacy of several pharmacotherapeutic interventions in osteoarthritic knee pain[59]. Among 63 RCTs assessing different interventions, 7 assessed GS and 6 CS, with minimal daily administered doses of 1500 mg and 800 mg, respectively. Mean pain relief values for GS or CS had no clinical relevance within 4, 6, 8 or 12 wk. Only for CH sulphate, there was a slight increase in efficacy equivalent to a categorical shift from none to perceptible improvement up to 12 wk.

A SR conducted by Lee *et al*[60] included six trials evaluating the effects of CH (4 studies) or GL (2 studies) on narrowing of joint space. They found significant small to moderate protective effects on minimum joint space narrowing, after 3 years of treatment with GS (SMD 0.43, 95%CI: 0.24-0.63, P < 0.001). The same was observed for CH sulphate, which had a small but significant protective effect on minimum joint space narrowing after 2 years (SMD 0.26, 95%CI: 0.13-0.39). This SR concluded that GL and CS may delay radiological progression of OA of the knee after daily administration for over 2 or 3 years. However, the number of RCTs assessed was low and important big studies were missing from the evaluation[61,62]. No clinical assessment was included in the outcomes and no methodological assessment of the included trials was performed. Two of the publications assessing CH where part of the same study, which was not taken into account in the meta-analysis[63,64].

A comprehensive Cochrane SR assessed RCTs of GL[12]. After the update in 2009, 25 RCTs were included (with 4963 patients). The analysis of the literature in this SR showed controversial results. There was evidence to show that GL is more effective in treating pain when compared with placebo showing an estimated relative per cent change from baseline of 22%. There was also superiority in Lequensne Index score (11% relative change from baseline), WOMAC total score and physician global assessment but not in other outcomes like WOMAC pain, stiffness and function subscales, minimum joint space width, patient global assessment. The majority of studies included had some form of relationship with a specific pharmaceutical manufacturer (Rottapharm). Interestingly, the authors found significant differences between the studies related with this manufacturer and the rest of the studies. Thus, studies in which this company's product was compared with placebo showed superiority of GL, even in radiological progression. However, pooled results from studies not using this product or from higher quality studies (with adequate allocation concealment) failed to show any benefit. It was clear though that GL had an

excellent safety profile, with complication rate equal to placebo and significantly less than NSAIDS.

Similarly, a recent SR from Singh *et al*[65], in the Cochrane library, included 42 RCTs that assessed the effectiveness of CH compared with placebo or control treatments. The authors concluded that there was a superiority of CH (alone or in combination with GL) over placebo, in terms of pain relief, in short-term studies. Moreover, CH had a lower risk of adverse events compared with control treatments. A limitation was the generally poor quality of studies available.

Regarding safety, all the SRs confirmed the safe profile of both GL and CH. In the total number of adverse events, withdrawals, or serious adverse events, no difference was found comparing with placebo[10,11,56]. Between trial heterogeneity, when reported for adverse events, was low in all cases[10,11].

DISCUSSION

There are several publications, from case series to RCTs, assessing the effectiveness and safety of GL and CH for the treatment of OA. However, there is criticism regarding the quality and validity of the majority of these studies. Even higher quality level I trials have been criticized for their non-transparent and low quality design. The vast majority have also been conducted by the manufacturing companies, increasing the risk of sponsorship bias. The low number of participants, non-defined source and preparation of the supplements used, short-term of follow up and outcome retrieval, non-defined dosing have also been discussed as sources of bias. Besides, there is increased heterogeneity among trials, mainly due to different dosing, different duration of application, different follow-up times, use of various escape or concomitant treatments (*e.g.,* pain killers, NSAIDS, physiotherapy).

Meta-analysis is the best tool available to collect and summarize all this spare and controversial information and to synthesise it, providing a more secure conclusion on the efficacy and safety of these interventions. The stratified analysis and subgroup analysis give the possibility to detect the effect of factors that are considered to potentially introduce heterogeneity or bias, like sponsorship of the study, inadequate treatment concealment, not binding of the outcome assessors, *etc*.

There are several level I SRs assessing GL and CH. Each of these has different inclusion or exclusion criteria resulting in a variety of number of studies included. The outcomes that are extracted from primary studies and analysed in the meta-analysis also differ in their nature and also in the time points assessed.

Despite the different methodology of these SRs, it seems that almost all conclude to a similar result; CH and GL have an effect size slight better when compared with placebo. However, when only the information from best quality trials are considered, then none of these supplements seem to demonstrate any superiority. Therefore, almost all of these level I reviews conclude to a lack of established efficacy, eventually suggesting that CH or GL should not be used in new patients.

Most of these SRs confirmed that the heterogeneity among trials could not be expected by chance alone. Bigger, methodologically sound independent trials did not show heterogeneity and did also not show relative efficacy of the intervention (either GL or CH)[10]. Cumulative analysis has also shown that newer publications showed smaller effects than did older publications[10,19].

According to the outcome of most of the SRs, there is a substantially increased risk of sponsorship bias in the available RCTs and this bias contributes to increased heterogeneity. It seems that the majority of the studies is financially supported in any form; either the manufacturer conducted the study, or provided with the drug or authors were supported. Sponsored trials showed more favourable results for the interventions although the rest of the studies did show null efficacy. It was also shown from some SRs that when a specific company was involved, the results were more favourable for the intervention. However, we should not exclude the possibility that some of this heterogeneity could be due to the use of different supplement formulations or to different dosing protocols. Such information was not regularly provided so to systematically detect this possibility.

Assumptions about reasons for failure

Animal studies have shown very good results favouring these supplements. However, it seems that these findings do not correlate with clinical level I studies. There are two possible explanations for this inconsistency. One might be the publication bias. It has been shown that studies with negative results are more likely not to be published[66,67]. This may be even more exacerbated in experimental animal studies, as usually protocols are not preregistered and therefore there is usually no obligation to publish any of the results. Another important reason is potentially the concentrations of supplements experimentally used in animals. The plasma concentrations achieved in animal studies can be hundreds times higher than the maximal concentration that can realistically be achieved after oral administration of 1500 mg of GL sulphate in human subjects[68]. Therefore, although a positive effect is noticed even in histological examination of cartilage, such a result cannot realistically be expected for humans[69]. It has been suggested that the therapeutic doses used in humans do not even allow the identification of proteoglycan synthesis as a mechanism of action of GL[69-71]. Therefore, extrapolation of the *in vitro* data directly to the *in vivo* situation should be done with great caution[69].

Pharmakokinetic and bioavailability of these supplements in the human joints after oral administration is certainly an issue that has to be further investigated[72]. There is evidence supporting that both GL and CH reach and retain a certain concentration in plasma

and also in joint fluid and cartilage, after normal doses administered *per os*[50,68,73-75]. However, as previously mentioned, there is no solid evidence to directly prove cartilage synthesis or regeneration in humans, as a result of this concentration.

Regarding dosing, little research has been published, thus no dietary reference intake currently exists for either GL or CH. There is an accepted daily dosage of 1500 mg for GL and 1200 mg for CH, rather empirically adopted, although different dosage schemes have been suggested in the literature[61,76]. This lack of consensus regarding the total daily dose or the dosing scheme may be an additional reason for the controversial and heterogeneous outcomes of related studies. However, the results and conclusions for the effectiveness or safety of GL and CH remain the same, even in SRs that excluded the subtherapeutic doses of GL and CH, which probably rejects this assumption[11,59].

A very important factor in the use of GL or CH is the length of therapy[46]. There are preliminary studies that showed clinical efficacy even at 4-12 wk of treatment[77,78]. However, these studies were of poor quality and high risk of bias and usually involved a rescue treatment with pain killers[46]. In more recent and higher quality trials, effects are not seen before 3 to 6 mo. Nevertheless, in most of the recent studies, the duration has been extended at least to 6 mo.

The selection of the patients and the use of treatment algorithms are probably mandatory. Even in single trials, there is usually not a limitation in specific age groups or OA grading. In 2 years follow up of GAIT trial, patients with more primary OA (Kellgren/Lawrence grade 2), seemed to have the higher potential for disease modification when compared with grade 3 cases, after combined GL and CH administration[62]. However, there is little known for the relative efficacy of any of these supplements in different age groups or different OA grades. Summarizing the outcomes of all these groups includes the assumption of equal action and effectiveness, which is yet not shown.

Felson *et al*[79] highlighted the role of the mechanical environment of an osteoarthritic joint for the success of any pharmacological treatment. Mechanical abnormalities, including joint malalignment, bony remodelling or instability, contributing to or being caused by the OA, may need to be addressed and corrected if possible, before any pharmacological treatment. None of the currently available drugs or supplements could probably have a reversible effect on the joint as a whole. Tissue-level dynamic stresses on cartilage in OA joints may also exceed thresholds that could be reversed by any effective pharmacologic agent. The mechanical factor has not been widely considered in the trials that assess the treatment role of either GL or CH, and this is potentially a reason for the lack of efficacy as it is shown in these trials.

Joint space narrowing has been used as an indicator for the alteration of the OA progression in the knee joint after the use of GL or CH[63,64,80-82]. Meta-analysis of this data has concluded that GL and CH may reduce the joint space narrowing after 2-3 years of continuous administration[60]. The SR of Wandel *et al*[11] additionally analysed 3 more recent RCTs concluding to a null effect size[11,62,83,84]. However, the measurement of joint space was performed by X-rays, which is criticised as a not accurate and reliable tool. In none of these studies the cartilage width was assessed.

Limitations of evidence

The quality and validity of the information provided above, regarding the efficacy and safety of GL or CH, is limited by the quality of the studies available. The low quality of published studies and the high risk of bias which is introduced by several factors (*e.g.*, poor methodology, poor reporting) limit the value of any suggestion or guidelines. The high interest of industry may have potentially impacted the currently available information.

There is evidence from funnel plots suggesting an absence of trials with both small numbers of participants and small or null treatment effects. This may be the result of selective publication of "positive" trials (that favours the new intervention) or of premature termination of trials with negative or null results. The high rate of sponsorship among the RCTs of GL or CH strengthens the possibility of high publication bias. However, this is just an assumption and in any case cannot be considered as evidence.

The pooling of different preparations of these supplements or products with different administration paths may increase the heterogeneity and decrease the validity of the outcomes in any meta-analysis. In many published trials the specific preparation of the supplements is not reported.

In many published meta-analyses, although the overall summary suggested a superiority of the intervention, the subgrouping of higher quality studies revealed a null effect size. In almost all cases only a few studies were of high quality. Therefore, one should argue that the limited number of studies decrease the power of the meta-analysis. This might provide a potential explanation for the trend for null effect sizes in such assessments.

Implications for research

Despite the large number of the available RCTs, there are still several questions not yet answered, first being the efficacy of GL and CH.

There is need for higher quality of information, either from RCTs or SRs. Therefore, more independent (not sponsored) high-quality randomized trials should be conducted. Trials should adhere to methodological standards that aim to reduce the risk of bias introduced (*e.g.*, CONSORT)[85]. SRs play also a mandatory role in evidence based information and should also follow similar standards (*e.g.*, MECIR)[86].

The best dosage scheme is still not yet defined by evidence. The duration of treatment that might provide

(if any) symptoms' relief or cartilage restoration is also still unknown. More advanced tools (*e.g.*, MRI) should be used to assess the joint and to detect for any restoration or regeneration of cartilage. The quality and quantity of cartilage should also be more accurately defined (*e.g.*, with DGEMRIC)[87].

It is still unclear which patients groups (if any) may profit from the use of such supplements. For this reason research, should be focused on assessing specific age groups, with specific OA grading. Inclusion criteria should be carefully and strictly defined. Idiopathic OA patients should be examined separately from secondary cases. By adding confounding factors like different stages of OA or different age groups the heterogeneity is increased, thus limiting the validity of outcomes. A more specific determination of supplements' characteristics and preparations is also mandatory to decrease this heterogeneity.

Implications for practice

There is currently no convincing information on the efficacy of GL or CH as treatment options in OA.

A positive effect of GL and CH on both clinical and radiological findings has been shown. However, only a few high-quality level I trials exist, especially for the assessment of radiological progression of OA. The effect sizes are small and probably not clinically relevant. However, even the validity of these results is limited by the high risk of bias introduced in the studies. Both GL and CH seem to be safe with no serious adverse events reported.

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