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Glucosamine and chondroitin use in canines for osteoarthritis: A review

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Abstract
Osteoarthritis is a slowly progressive and debilitating disease that affects canines of all breeds. Pain and decreased mobility resulting from osteoarthritis often have a negative impact on the affected canine's quality of life, level of comfort, daily functioning, activity, behaviour, and client-pet companionship. Despite limited and conflicting evidence, the natural products glucosamine hydrochloride (HCl) and chondroitin sulfate are commonly recommended by veterinarians for treating osteoarthritis in dogs. There is a paucity of well-designed clinical veterinary studies investigating the true treatment effect of glucosamine and chondroitin. The purposes of this review article are to provide a brief background on glucosamine and chondroitin use in canine osteoarthritis and to critically review the available literature on the role of these products for improving clinical outcomes. Based on critical review, recommendations for practice are suggested and a future study design is proposed.
Keywords: Canine, Chondroitin, Glucosamine, Osteoarthritis, Veterinary.

Introduction

Osteoarthritis is a slowly progressive, degenerative, and debilitating disease affecting 20% of the canine population over the age of one (Johnston, 1997; Johnson *et al*., 2001; Roush *et al*., 2002; Aragon *et al*., 2007). Large-breed dogs may develop more severe clinical signs and initial symptoms of osteoarthritis; however, dogs of all sizes and breeds are affected by the disease as they age (Rychel, 2010).

The etiology of osteoarthritis' pathology may include defective articular cartilage structure, inadequate cartilage biosynthesis, joint trauma, instability, and inflammatory mechanisms. The disease presents with symptoms such as pain, stiffness, lameness, and disability (D'Altilio *et al*., 2007).

Pain and decreased mobility resulting from osteoarthritis often have a negative impact on the affected canine's quality of life, level of comfort, daily functioning (i.e. standing, walking), exercise tolerance, activity (i.e. playing, climbing stairs), behaviour, urinary and fecal habits, and client-pet companionship. Owners of severely affected dogs may decide to euthanize their pet (Rychel, 2010; Epstein *et al*., 2015). Once a canine develops osteoarthritis, exploring treatment options becomes essential for minimizing the negative consequences of the disease. Non-pharmaceutical treatment options may include surgery, weight loss, exercise modification, and physical therapy (Beale, 2004).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the current gold-standard pharmaceutical therapy for dogs with osteoarthritis; however, NSAIDs may cause gastrointestinal ulceration as an adverse effect and are contraindicated in the presence of renal insufficiency or dehydration. Other pharmaceutical options include diacerhein, corticosteroids, and hyaluronic acid (Henrotin *et al*., 2005). Select nutraceuticals such as glucosamine, chondroitin, pentosane polysulphate, avocado/soybean unsaponifiables, green-lipped mussel, and milk protein have also been used (Henrotin *et al*., 2005).

Glucosamine hydrochloride (HCl) and chondroitin sulfate (CS) are commonly recommended natural health products for treating osteoarthritis in dogs (Rychel, 2010). Glucosamine regulates the synthesis of collagen in cartilage and may provide mild anti-inflammatory effects while chondroitin sulfate inhibits destructive enzymes in joint fluid and cartilage. The two nutraceuticals also contribute to the synthesis of glycoaminoglycans and proteoglycans, which are building blocks for the formation of cartilage (Beale, 2004).

In humans, glucosamine is available in several dosage forms; glucosamine hydrochloride (HCl), glucosamine sulfate (stabilized with different salts, usually potassium chloride) and crystalline glucosamine sulfate. N-acetyl glucosamine is another available salt form, but it appears to have no clinical activity as compared to the other salt forms (Beale, 2004).

In terms of efficacy, crystalline glucosamine sulfate has shown the greatest efficacy for osteoarthritis of the knee which is likely due to an improved oral bioavailability (25%-44%) as compared to other glucosamine salts (Setnikar and Rovati, 2001; Persiani

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et al., 2005; Altman, 2009). Crystalline glucosamine sulfate is a pharmaceutical-grade prescription product in Europe (but not in the United States or Canada) that consists of glucosamine, sulfate, sodium and chloride ions in a specific stoichiometric ratio (Altman, 2009). The other glucosamine salt forms (HCl, sulfate salts) have demonstrated variable efficacy in humans (Sawitzke *et al*., 2010; Rovati *et al*., 2012). This is primarily due to inconsistency in glucosamine content amongst nutraceutical products and poor oral bioavailability, especially in combination with other nutraceutical additives (Altman, 2009; Sawitzke *et al*., 2010; Wandel *et al*., 2010).

Similar to human products, there are various manufactured glucosamine and chondroitin products marketed for canines that differ in terms of strength, formulation, and additional active ingredients. Table 1 provides reference to various examples of glucosamine and chondroitin products marketed for canines. It should be noted that the majority of veterinary supplements contain glucosamine HCl, which is already known to have poorer bioavailability and poor clinical effect in humans.

There are several hypothesized reasons for this salt choice in veterinary products. First, the hydrochloride salt from a chemical perspective provides a greater amount of glucosamine per gram than does the sulfate salt despite the fact previous studies report overall lower oral bioavailability (Beale, 2004). The sulfate salt is often stabilized with sodium chloride (NaCl) or potassium chloride (KCl), which may be undesirable in aging canines with potential co-morbid medical conditions such as heart failure, hypertension or renal decline. Although this is a theoretical concern, human clinical trials have not demonstrated increases in blood pressure with NaCl content of crystallized glucosamine sulfate (Herrero-Beaumont *et al*., 2007; Rovati *et al*., 2012).

Last, the hydrochloride salt is much cheaper to produce; keeping in mind that crystalline glucosamine sulfate is manufactured as pharmaceutical-grade with strict quality control standards (Altman, 2009).

There is currently a lack of evidence to confirm a specific therapeutic dose of glucosamine in canines, yet, an adjunctive chondroitin dose of 15-30mg/kg has been suggested (Plumb, 2015). Few *in vitro* studies have provided bioavailability and pharmacokinetic data differentiating the most optimally absorbed glucosamine formulation in canines.

In horses, crystalline glucosamine sulfate achieves higher concentrations than glucosamine HCl (Meulyzer *et al*., 2008). One study in dogs demonstrated oral bioavailability of 12% and 5% for glucosamine hydrochloride and chondroitin sulfate respectively. (Adebowale *et al*., 2002).

Table 1. Examples of Nutraceutical Products Marketed for Canines with Osteoarthritis (Henrotin *et al*., 2005) and their Various Ingredients.

Propriety Name	Containing Ingredients/Tablet
ProMotion for Medium Large Dogs (PetMed Express Inc., 2016).	Glucosamine HCl 700 mg, Manganese 10 mg, Zinc 2 mg, Ascorbic Acid 25 mg, Cysteine 25 mg.
Dasuquin with MSM (Nutramax Laboratories Veterinary Sciences Inc., 2016b).	Large Dogs: Glucosamine HCl 900 mg, 350 mg CS, 90 mg Avocado/Soybean Unsaponifiables, 800 mg MSM. Small Dogs: Glucosamine HCl 600 mg, 250 mg CS, 45 mg Avocado/Soybean Unsaponifiables, 400 mg MSM.
Glyco-Flex III Soft Chews (Vetri-Science Laboratories, 2016).	Glucosamine HCl 1000 mg, MSM 1000 mg, Green Lipped Mussel 600 mg, DMG 100 mg, dl-alpha Tocopheryl Acetate 50 IU, Calcium Ascorbate 30 mg, Ascorbic Acid 24 mg, Mg 10 mg, Grape Seed Extract 5 mg, L-Glutathione 2 mg.
TerraMax Pro Hip & Joint Supplement (TerraMax Pro, 2016).	1600 mg Glucosamine HCl, 1200 mg Chondroitin Sulfate, 1000 mg Opti-MSM.
Extend K9 Health Formula Joint Care (Extend Joint Care, 2016).	Glucosamine HCl 300 mg, MSM, Type II Collagen, and Ascorbic Acid 400 mg, other quantities not specified.
Pet Naturals Hip & Joint Tablets (Pet Naturals of Vermont, 2016).	750 mg Glucosamine HCl, 400 mg Chondroitin Sulfate, MSM 400 mg, Ascorbic Acid 100 mg, Magnesium Proteinate 5 mg.
Cosequin DS (Nutramax Laboratories Veterinary Sciences Inc., 2016a).	Glucosamine HCl 500 mg, Chondroitin Sulfate 500 mg, Manganese 3 mg.
Liquid Health K9 Glucosamine (Liquid Health Inc., 2016).	Glucosamine HCl 1600 mg, CS 1200 mg, MSM 1000 mg, Manganese Chelate 7 mg, Hyaluronic Acid 10 mg.

(CS): Chondroitin sulfate; (DMG): Dimethylglycine; (HCL): Hydrochloride; (IU): International units; (MSM): Methyl-sulfonyl-methane.

Some studies have indicated that when administered to dogs as a combination, glucosamine and chondroitin are absorbed in as little as two hours (Beale, 2004). One commentary notes that glucosamine HCl and chondroitin sulfate require 10 to 20 times the quantity used in *in vitro* studies to reach a plasma concentration that will result in biological activity (Comblain *et al*., 2016).

It has been suggested that 2-6 weeks of treatment with glucosamine and chondroitin may be necessary for any therapeutic effect to become apparent (Plumb, 2015), but there is a lack of clinical evidence to support this statement. Potential adverse effects include hypersensitivity and minor gastrointestinal effects such as flatulence and stool softening (Plumb, 2015).

Veterinarians commonly recommend glucosamine and chondroitin for treating osteoarthritis in canines despite the lack of compelling scientific evidence demonstrating clinical benefit.

Clinical trials to date have used different products, salt forms, doses, and dosing regimens such that comparing the results to draw meaningful conclusions about therapeutic efficacy is difficult (Addleman, 2010). In addition, pharmacists are often approached by pet owners with questions about the use of over-the-counter natural products in pets due to the availability of these products in pharmacies.

Unfortunately, the lack of high-quality research on natural product use in pets makes it difficult to offer informed recommendations to pet owners with regard to glucosamine and chondroitin.

The purpose of this review is to critically appraise the available literature on the role of glucosamine and chondroitin in improving clinical outcomes in canines with osteoarthritis. We will propose evidence-based recommendations for practice and provide suggestions regarding the design of future clinical studies.

Evidence summary

Clinical trial: Glucosamine and chondroitin versus NSAID or placebo

Moreau *et al*. (2003) conducted a prospective, randomized, double-blinded study including 71 client-owned dogs >12 months old and >20 kg with owner-reported lameness and radiographic signs of osteoarthritis.

The trial consisted of four arms in which the subjects received either: 1) glucosamine HCl, chondroitin sulfate, and magnesium ascorbate (GSCM), 2) carprofen, 3) meloxicam, or 4) placebo. For complete dosing and titration schedules, please see Table 2.

Primary outcomes included treatment efficacy, tolerability and ease of administration. Efficacy was measured objectively through ground reaction force (GRF) values and subjectively through owner and orthopaedic surgeon assessments at 0, 30 and 60 days of treatment. Blood and faecal analyses were conducted on the same schedule to determine treatment safety. The placebo and GCSM arms did not experience statistically significant improvements in any of the outcome measures by trial end.

In contrast, both NSAID arms experienced significant improvements in GRF values and orthopaedic surgeon assessment scores; however, only the meloxicam arm experienced a significant improvement according to owner assessment.

The Moreau *et al*. (2003) trial had several strengths. The study was double-blinded, prospective and subjects were randomized to treatment groups. Additionally, the authors claimed that mean age, weight, affected limb GRF values, radiographic scores, and subjective scores of the dogs in the four study arms were all similar at baseline, although data to support this claim was not provided. Weaknesses of the trial included that glucosamine and chondroitin doses are much lower in comparison to other clinical trials and the treatment regimens differed between study arms.

The meloxicam arm received a loading dose, the GCSM dose was decreased over the course of the trial, and the placebo arm was discontinued after 30 days while all other interventions continued for 60 days. While the GCSM arm did not experience any significant outcome improvements by trial end, it is possible that the intervention was ineffective due to the absence of a GCSM loading dose, the use of sub-therapeutic GCSM doses throughout the trial, and/or an insufficient trial length. The fact that the improvement in GRF values experienced by the carprofen arm was not accompanied by an improvement in subjective owner assessment scores questions the clinical significance of GRF values. Eight of the 71 subjects (11.3%) were lost to follow-up and the authors did not disclose which study arms were affected by dropout.

Mean assessment scores with confidence intervals for GRF, orthopaedic surgeon assessment and owner assessment were not provided. The primary outcome stated by investigators was to identify the "best" treatment for dogs with osteoarthritis which requires appropriately designed statistical methods to compare treatment arms. However, statistical comparisons and treatment rankings were not provided and the magnitudes of the treatment effects were not reported.

Clinical trial: glucosamine and chondroitin versus placebo or NSAID

Investigators in the McCarthy *et al*. (2007) group conducted a prospective, randomized, double-blinded study that included 42 client-owned dogs, with 35 completing the trial. The dogs could be of any breed or sex, presenting with clinical signs of chronic lameness, stiffness, joint pain, and radiological evidence of osteoarthritis of the hips and/or elbows. The trial consisted of two arms in which the subjects received either: 1) glucosamine HCl, chondroitin sulfate, N-acetyl-D-glucosamine, ascorbic acid, and zinc sulfate or 2) carprofen. For complete dosing and titration schedules, please see Table 2. The primary outcome of efficacy in the treatment of osteoarthritis was determined through subjective veterinarian assessment at 0, 14, 42, 70 and 98 days of treatment.

Table 2. Literature Overview on Glucosamine and Chondroitin Use in Canines for Osteoarthritis.

Reference	Design, Subjects, & Duration	Intervention(s)	Findings/Results
Systematic Reviews			
Aragon *et al*. (2007)	Included 1 trial: - Moreau trial.	See Moreau trial summary below.	No subjective or objective improvements in comparison to placebo. Insufficient design quality for generalizability.
Vandeweerd *et al*. (2012)	Included 2 trials: - McCarthy trial. - Moreau trial.	See McCarthy & Moreau trial summaries below.	Trials used different compounds and had conflicting results. The McCarthy trial showed beneficial effects while the Moreau trial showed no effect; however, the Moreau trial used a combination of GHCl + CS + MA. Efficacy evidence is of low quality and MA may have contributed to the results.
Clinical Trials			
Moreau *et al*. (2003)	***Design:*** Prospective, randomized (via computer-generated list), double-blinded trial ***Subjects:*** 71 client-owned dogs with OA who were >12 months old and >20 kg with chronic and stable lameness reported by the owner plus radiographic signs of OA in one or two elbows, stifles, or hips; compared to pure-breed dogs with normal GRF measurements ***Exclusion criteria****:* Pregnancy; hypersensitivity to NSAIDs; neurological or musculoskeletal pathology; orthopaedic surgery within the same year; gait abnormalities involving both hind and fore limbs; concurrent osteoarthritis treatment ***Outcomes****:* Efficacy, tolerance, and ease of administration, measured at days 30 and 60 ***Objective outcome measures:*** GRF measurements (provided data about the level of pain-related functional impairment present) ***Subjective outcome measures:*** Gait, articular mobility, articular pain and discomfort (indicated by vocalization), lameness, and activity ***Duration:*** 30 or 60 days	***Number of study arms:*** Four ***Intervention:*** GHCl 500 mg + CS 400 mg + MA 75 mg dosed as either 2 caps AM and 1 cap in the afternoon for 30 days followed by 1 cap q12h for 30 days if <45 kg or 2 caps BID for 30 days followed by 2 caps AM and 1 cap at noon for 30 days if >45 kg ***Comparator arms:*** 1) Carprofen 2.2 mg/kg q12h for 60 days 2) Meloxicam 0.2 mg/kg for the first day followed by 0.1 mg/kg for 59 days 3) Placebo for 30 days	***Efficacy:*** The GHCl + CS + MA and placebo arms did not experience statistically significant improvements in any of the outcome measures by trial end. The carprofen arm experienced statistically significant improvements in GRF values and orthopaedic surgeon assessment scores, but not in subjective owner assessment scores. The meloxicam arm experienced statistically significant improvements in GRF values, orthopaedic surgeon assessment scores, and owner assessment scores ***Safety:*** One dog in the meloxicam arm experienced vomiting. One dog in the carprofen arm experienced anorexia, lethargy, jaundice, and vomiting and was diagnosed with toxic idiosyncratic hepatitis to carprofen. Both dogs were withdrawn from the trial.

Table 2: Literature Overview on Glucosamine and Chondroitin Use in Canines for Osteoarthritis (*Cont.*).

McCarthy *et al.* (2007)	***Design:*** Multi-centered, prospective, randomized (alternating order of enrollment), double-blinded trial. ***Subjects:*** 42 client-owned dogs of any breed or sex presenting with clinical signs of chronic lameness (present for at least 1 month), stiffness, joint pain, and radiological evidence of OA of the hips and/or elbows; 35 completed the trial. ***Exclusion criteria:*** Pregnancy; current use of other medications; hepatic, renal, and/or CV disease; gastrointestinal ulceration; bleeding disorder; lameness due to infectious, immune-mediated, neurological, or neoplastic disease; previous use of drugs and/or dietary supplements for the treatment of OA. ***Outcomes:*** Efficacy in the treatment of confirmed OA, measured at days 14, 42, and 70; additionally, compliance was assessed by counting the number of capsules remaining at each visit. ***Subjective outcome measures****:* Scores for lameness, joint mobility, pain on palpation, weight-bearing, and an overall score for clinical condition; severity of condition, subjective veterinarian evaluation, and withdrawal symptoms were also measured. ***Duration:*** 70 days.	***Number of study arms:*** Two ***Intervention:*** GHCl 475 mg/g, CS 350 mg/g, NADG 50 mg/g, AA 50 mg/g, and ZS 30 mg/g with total doses of 1 g, 1.5 g, or 2 g of active ingredient BID for 42 days for dogs weighing 5-19.9 kg, 20-40 kg, or >40 kg respectively, followed by a dose decrease by one-third of the original dose for the subsequent 28 days; administered with food. ***Comparator arm:*** Carprofen 2 mg/kg BID for 7 days followed by 2 mg/kg SID for the subsequent 63 days; administered with food.	***Efficacy:*** The GHCl + CS + NADG + AA + ZS arm showed statistically significant improvements from baseline with regard to pain, weight-bearing, and overall condition scores at 70 days. Lameness and joint mobility scores did not improve significantly by trial end. The carprofen arm showed significant improvements from baseline with regard to all five parameters at or before 70 days. ***Safety:*** Two dogs in the GHCl + CS + NADG + AA + ZS arm experienced unspecified adverse drug reactions and were withdrawn from the trial.
Gupta *et al.* (2012)	***Design:*** Prospective, randomized, controlled, double-blinded trial. ***Subjects:*** 31-37 client-owned dogs (each of the four trial arms consisted of 7-10 dogs) weighing >40 lbs with moderate OA. ***Exclusion criteria:*** Serious concomitant diseases or complications. ***Outcomes:*** Therapeutic efficacy, tolerability, and safety, measured on a monthly basis. ***Objective outcome measures:*** Peak vertical force and impulse area measurements obtained with a piezoelectric sensor-based ground force plate (indicators of lameness due to pain); physical, hepatic, and renal functions were monitored via body weight, temperature, pulse, ALP, ALT, bilirubin, BUN, and Cr measurements. ***Subjective outcome measures:*** Overall pain, pain upon limb manipulation (vocalization), pain after physical exertion (limping and limb rigidity), signs of pain, signs of lameness, severity of pain during various activities (i.e. playing), and overall performance assessments (running, participation in activities, movement, change between sitting and standing). ***Duration:*** 150 days.	***Number of study arms:*** Four ***Interventions:*** 1) GHCl 2000 mg + CS 1600 mg + UCII 10 mg given daily 2) GHCl 2000 mg + CS 1600 mg given daily 3) UCII 10 mg given daily ***Comparison arm****:* Placebo given daily.	***Efficacy:*** The placebo arm did not experience statistically significant changes in any of the outcome measures by trial end. The GHCl + CS arm exhibited a significant reduction in pain by day 90 with maximal effects observed on day 150. Specifically, overall pain had decreased by 51%, pain after limb manipulation had decreased by 48%, and pain after physical exertion had decreased by 43% from baseline at 150 days. Ground force plate-based parameters remained significantly unchanged by trial end. Supplementing GHCl + CS with UCII did not provide any additional benefit. ***Safety:*** None of the dogs receiving dietary supplements showed any signs of adverse effects.

Table 2. Literature Overview on Glucosamine and Chondroitin Use in Canines for Osteoarthritis (*Cont.*).

D'Altilio *et al*. (2007)	***Design:*** Prospective, randomized, controlled, double-blinded trial. ***Subjects****:* 20 client-owned dogs presenting with joint stiffness, lameness, moderate pain, swollen joints, difficulty getting up/down, and difficulty walking in horizontal areas or stairs due to OA. ***Outcomes:*** Therapeutic efficacy and safety, measured on a monthly basis. ***Objective outcome measures:*** Body weight, hepatic function (ALT, bilirubin), and renal function (BUN, Cr) were measured to monitor for adverse effects. ***Subjective outcome measures****:* Overall pain (trouble changing between sitting and standing, vocalization, crying), pain upon limb manipulation (vocalization), and exercise-associated lameness (limping, holding limb up, limb rigidity). ***Duration:*** 120 days of intervention exposure followed by a 30-day withdrawal period.	***Number of study arms:*** Four ***Interventions:*** 1) GHCl 2000 mg + CS 1600 mg + UCII 10 mg given daily 2) GHCl 2000 mg + CS 1600 mg given daily 3) UCII 10 mg given daily ***Comparison arm:*** Placebo given daily	***Efficacy:*** The placebo arm did not experience statistically significant changes in any of the outcome measures by trial end. The GHCl + CS arm experienced a reduction in pain that was not significant and showed relapse following the 30-day treatment withdrawal period. Supplementing GHCl + CS with UCII did reduce overall pain, pain upon limb manipulation, and exercise induced lameness to a significant extent, although this benefit was also lost following the 30-day treatment withdrawal period. ***Safety:*** None of the dogs receiving dietary supplements showed any signs of adverse effects.
In Vitro Studies			
Anderson *et al*. (1999)	N=2 adult female dogs recently euthanized for reasons unrelated to orthopedic abnormalities. Measured chondrocytes for viable cells, PGE2 and GAG concentrations at days 3, 6, and 12.	***Number of study arms:*** Three ***Interventions:*** 1) Chondrocytes cultured in glucosamine 100 mcg/mL. 2) Chondrocytes cultured in acetylsalicylate 18 mcg/mL. 3) Chondrocytes cultured in a control medium.	Chondrocytes in all three mediums had characteristics indicative of viability and differentiation.
Adebowale *et al*. (2002)	Randomized three-way single dose cross-over study and multiple dose open study. N=8 male beagle dogs of age >6 months weighing approximately 9 kg. Bioavailability and pharmacokinetics of single and multiple doses were measured through blood and plasma samples. A typical blood sampling scheme was measured pre-dose and at 0.5, 1, 2, 4, 6, 8, 10, 12, 14, and 24 hours following drug administration.	***Number of study arms:*** Four ***Interventions:*** 1) IV GHCl 500 mg + LMWCS 400 mg for 14 days. 2) PO GHCl 1500 mg + LMWCS 1200 mg for 14 days. 3) PO GHCl 2000 mg + LMWCS 1600 mg for 14 days. 4) PO GHCl 1500 mg + LMWCS 1200 mg on days 1-7 followed by PO GHCl 3000 mg + LMWCS 2400 mg on days 8-14.	GHCl and LMWCS are bioavailable after oral dosing. LMWCS results in significant accumulation upon multiple dosing. GHCl and LMWCS BA were 12% and 5%, respectively. Cmax=8.95 mcg/mL and Tmax=1.5 hours following 1500 mg dose of GHCl. Cmax=21.5 mcg/mL following 1600 mg dose of CS.

Table 2. Literature Overview on Glucosamine and Chondroitin Use in Canines for Osteoarthritis (*Cont.*).

Surrogate Outcome Trials			
Johnson *et al.* (2001)	N=16 pure-bred dogs weighing 23-32 kg with surgically-induced OA; not client-owned. Measured concentrations of synovial fluid markers at 0, 1, 3, & 5 months.	***Number of study arms:*** Four ***Interventions:*** 1) GHCl 250 mg + CS 200 mg + MA 5 mg + CCL reconstruction. 2) GHCl 250 mg + CS 200 mg + MA 5 mg + sham CCL reconstruction. 3) Sham CCL reconstruction 4) CCL reconstruction.	Heterogeneity of results from synovial fluid analyses reported. GHCl + CS + MA arms had significantly higher levels of beneficial synovial fluid markers; however, concentrations were not localized to joints.
Canapp *et al.* (1999)	N=32 skeletally mature mixed-breed dogs of age 1-5 years weighing 4.5-11 kg with chemically-induced synovitis. Measured SA at days 13, 20, 27, 34, 41, and 48 post-SI; measured lameness at days 1-48 post-SI.	***Number of study arms:*** Four ***Interventions:*** 1) GHCl 500 mg + CS 400 mg + manganese 10 mg + ascorbate 66 mg (GlAm-CS) q8h for 21 days pre-SI, then GlAm-CS for 48 days post-SI. 2) Placebo for 21 days pre-SI, then GlAm-CS for 48 days post-SI. 3) Placebo for 21 days pre-SI, then GlAm-CS + SAMe 200 mg for 48 days post-SI 4) Placebo for 21 days pre-SI, then placebo for 48 days post-SI.	Dogs given pre-SI GlAm-CS showed significantly less soft-tissue SA at day 48 and significantly less bone SA at days 41 and 48 compared to the other study arms, with less SA being suggestive of a protective effect against synovitis. Dogs given pre-SI GlAm-CS showed a significant decrease in lameness on days 12, 19, 23, and 24 compared to the other study arms. Significant differences in SA and lameness were not found at any time among the study arms that did not receive pre-SI therapy.
Review Articles			
Pascoe (2002)	Not applicable.	Glucosamine & chondroitin.	Article reviews studies in humans, horses, and dogs.
Henrotin *et al.* (2005)	Not applicable.	Glucosamine sulfate & CS.	*In vitro* studies show increased production of proteoglycans by chondrocytes; however, results cannot be extrapolated to different preparations. No scientifically conducted trials demonstrate disease-modifying properties.
Johnston *et al.* (2008)	Not applicable.	GHCl & CS.	Refers to Moreau and McCarthy trials. Concludes that based on the quality of the trials, one can be moderately comfortable with the results despite their lack of consistency.
Addleman (2010)	Not applicable.	Glucosamine & chondroitin.	Purity, quality, efficacy, dosing, and absorption of glucosamine and chondroitin vary and evidence is limited. There is a need for validated owner questionnaires, long-term studies with objective measures, and a better understanding of their mode of action.
McKenzie (2010)	Not applicable	Glucosamine & chondroitin	The evidence is limited in terms of quantity and quality and the results are mixed.

Table 2. Literature Overview on Glucosamine and Chondroitin Use in Canines for Osteoarthritis (*Cont.*).

KuKanich (2013)	Not applicable.	Glucosamine & chondroitin dosed q24h.	Current literature does not support the use of glucosamine and chondroitin for the control of osteoarthritis pain in dogs.
Comblain *et al.* (2016)	Not applicable.	Glucosamine & chondroitin.	Studies have contrasting results.
Neil *et al.* (2005)	Not applicable.	Glucosamine & chondroitin.	*In vitro* studies indicate rapid absorption, a good safety profile, and chondroprotective effects in dogs. Minimal effective concentrations of these compounds and beneficial effects in dogs require further investigation.

(AA): Ascorbic acid; (ALP): Alkaline phosphatase; (ALT): Alanine transaminase; (AM): Morning, (BA): Bioavailability; (BID): Twice daily; (BUN): Blood urea nitrogen; (CCL): Cranial cruciate ligament; (Cmax): Maximum or peak serum concentration; (Cr): Creatinine; (CS): Chondroitin sulfate; (CV): Cardiovascular; (GAG): Glycosaminoglycan; (GHCl): Glucosamine hydrochloride; (GlAm-CS): Glucosamine and chondroitin sulfate; (GRF): Ground reaction force; (IV): Intravenous; (LMWCS): Low molecular weight chondroitin sulfate; (MA): Manganese ascorbate; (MSM): Methyl-sulfonyl-methane; (N): Number of study subjects; (NADG): N-acetyl-D-glucosamine; (OA): Osteoarthritis; (PGE2): Prostaglandin E2; (PO): By mouth; (q12H): Every 12 hours; (SAMe): S-adenosyl-L-methionine; (SA): Scintigraphic activity; (SI): Synovitis induction; (SID): Once daily; (Tmax): Time to reach maximum concentration; (UCII): Undenatured collagen type II; (ZS): Zinc sulfate.

The outcome measures included scores for joint mobility, lameness, pain on palpation, weight-bearing, and an overall score for clinical condition. In the carprofen arm, statistically significant improvements were found between the pre-treatment and change scores for all five efficacy parameters at or before 70 days. In contrast, the glucosamine and chondroitin arm showed statistically significant improvements in pain, weight-bearing, and overall condition for the first time at 70 days, while lameness and joint mobility did not improve to a significant extent by trial end. The authors also concluded that glucosamine and chondroitin therapy was non-inferior to carprofen therapy at day 70 in the treatment of osteoarthritis in dogs.

The McCarthy *et al.* (2007) trial was multi-centered, randomized, double-blinded, and prospective which is an ideal study design. However, the method of randomization was determined by order of presentation (alternating), which introduces the risk of selection bias since the ability to anticipate treatment allocation may potentially influence the order of enrollment. Baseline characteristics of the two groups of canines had some variation in terms of mean weight, age, and affected joints.

Therapeutic efficacy scores were based on subjective assessments conducted by veterinarians, which could be highly variable between clinicians. Six dogs from the glucosamine and chondroitin arm failed to complete the study, two of which were withdrawn due to experiencing unspecified adverse drug reactions. One dog from the carprofen arm failed to complete the study. With seven dropouts, the loss to follow-up was high (16.7%). The collected data underwent a per-protocol analysis (versus intention-to-treat) therefore we cannot comment on the robustness of the results. The reported result of therapeutic efficacy in the carprofen arm did support the validity of the results for the glucosamine and chondroitin arm. However, the absence of a comparator placebo arm in the study design calls into question whether the glucosamine and chondroitin arm was more or less effective as compared to placebo.

The authors claimed that glucosamine and chondroitin therapy was *non-inferior* to carprofen therapy at day 70. Non-inferiority studies often require large sample sizes and rigorous statistical methods to demonstrate non-inferiority between two treatments. The decision to use a sample size that provided 78% power to detect a difference in the median subjective veterinarian assessment score of one point was questionable and lacking in transparency of statistical method design. A median reduction of one point in the subjective veterinarian assessment score represented a clinically significant improvement in the canines' condition, but justification for selection of this score was absent.

Clinical trials: Glucosamine/chondroitin versus undenatured collagen type II, placebo, or glucosamine/chondroitin/undenatured collagen type II

Gupta *et al.* (2012) conducted a prospective, randomized, double-blinded study that included approximately 31-37 client-owned dogs weighing >40 lbs with moderate osteoarthritis. The trial consisted of four arms in which the subjects received either 1) glucosamine HCl, chondroitin sulfate, and undenatured collagen type II (UCII), 2) glucosamine HCl and chondroitin sulfate, 3) UCII, or 4) placebo. For complete dosing schedules, please see Table 2. Outcomes included therapeutic efficacy, tolerability, and safety. Efficacy was measured objectively through

peak vertical force and impulse area measurements obtained with a piezoelectric sensor-based ground force plate (GFP) and subjectively through observational pain assessments on a monthly basis for 150 days. Additionally, the physical, hepatic, and renal functions of the dogs were monitored each month via body weight, temperature, pulse, alkaline phosphatase (ALP), alanine transaminase (ALT), bilirubin, blood urea nitrogen (BUN), and creatinine (Cr) measurements. The placebo arm showed no statistically significant changes in any of the outcome measures by trial end. The glucosamine and chondroitin arm exhibited a significant reduction in subjectively-assessed pain at 90 days with maximal effects observed at 150 days. The GFP-based parameters remained significantly unchanged by trial end. Supplementing glucosamine and chondroitin with UCII did not provide any additional benefit. None of the dogs receiving dietary supplements showed any signs of adverse effects.

Strengths of the Gupta *et al*. (2012) trial were that it was prospective, randomized, controlled, and double-blinded. Weaknesses of the trial were that baseline patient characteristic information was not provided and the analysis protocol was vague.

Investigators in the D'Altilio *et al*. (2007) group conducted a prospective, randomized, double-blinded study that included 20 client-owned dogs with joint stiffness, lameness, moderate pain, swollen joints, difficulty getting up/down, and difficulty walking in horizontal areas or stairs due to osteoarthritis. The trial consisted of four arms in which the interventions were identical to those in the Gupta *et al*. (2012) trial. However, in contrast, intervention exposure lasted only 120 days followed by a 30-day withdrawal period. Outcomes included therapeutic efficacy and safety. Efficacy was measured subjectively through observational pain assessments on a monthly basis for 150 days. Additionally, body weight, hepatic function (ALT, bilirubin), and renal function (BUN, Cr) were measured each month to monitor for adverse effects. While the placebo arm exhibited no statistically significant changes in any of the outcome measures by trial end, the other results of the D'Altilio *et al*. (2007) trial differed from those of the Gupta *et al*. (2012) trial. The glucosamine and chondroitin arm showed a reduction in pain that was not significant and relapsed following the withdrawal of treatment for 30 days. As well, supplementing glucosamine and chondroitin with UCII did provide additional benefit to the point of reducing pain to a significant extent, although this benefit was also lost following the withdrawal of treatment for 30 days. None of the dogs receiving dietary supplements showed any signs of adverse effects. Strengths of the D'Altilio *et al*. (2007) trial were that it was prospective, randomized, controlled, and double-blinded. Weaknesses of the trial were that the baseline patient characteristics were unspecified and the follow-up and analysis protocol were unclear.

Surrogate endpoint/in vitro studies

In vitro studies investigating surrogate outcomes related to osteoarthritis treatment in dogs suggest that the use of glucosamine and chondroitin produces chondroprotective effects (Anderson *et al*., 1999). Currently, good-quality evidence does not exist to suggest that *in vitro* studies using surrogate endpoints translate into clinically meaningful improvements in canine osteoarthritis symptoms. Table 2 provides a brief summary of surrogate endpoint/*in vitro* trials for reader interest.

Review articles

Eight commentaries reviewing the evidence around glucosamine and chondroitin use in canines with osteoarthritis were available in the literature. These commentaries are presented below in chronological order from the time of publication.

The Pascoe (2002), Henrotin *et al*. (2005), and Neil *et al*. (2005) commentaries pre-date or opt not to discuss clinical trials investigating the use of glucosamine and chondroitin for pain reduction and improved mobility in canines. However, Pascoe (2002) notes that despite the lack of clinical evidence, 62% of surveyed veterinary practitioners reported recommending products containing glucosamine and chondroitin for canines because they believed that they were seeing beneficial effects with their use. Similarly, Henrotin *et al*. (2005) appear to give precedence to anecdote over scientific evidence by concluding that glucosamine and chondroitin have clearly demonstrated symptomatic action. Neil *et al*. (2005) concludes that the determination of the minimal effective concentrations of glucosamine and chondroitin and their beneficial effects in canines require further investigation.

The Johnston *et al*. (2008) commentary refers to the Aragon *et al*. (2007) systematic review, the Moreau *et al*. (2003) trial, and the McCarthy *et al*. (2007) trial. The authors conclude that despite having conflicting results, the two studies shared similar strengths such that one can have a moderate level of comfort with the results from both studies. In contrast, the Addleman commentary (Addleman, 2010) identifies the lack of high-quality clinical trials and objective measures of efficacy as well as the unknown absorption and duration of effect of nutraceuticals as limitations in the current evidence around glucosamine and chondroitin use in canines with osteoarthritis.

The author concludes that objective methods for measuring joint disease symptoms, mobility, and pain using force plate gait analysis, accelerometers, and validated pain scales need to be established and that effective glucosamine and chondroitin dosing needs to be determined using dogs as the study subjects, as

canine dosing is currently extrapolated from studies conducted in other species and therefore suboptimal. Similarly, the McKenzie (2010) commentary concludes that clinical trial evidence is severely limited. The author calls for veterinarians to translate the uncertainty around the usefulness of glucosamine and chondroitin therapy when discussing this treatment option with dog owners. McKenzie (2010) points out that there is a lack of literature addressing the use of glucosamine and chondroitin as an adjunct to NSAID therapy.

KuKanich (2013) commentary concludes that current literature does not support the use of glucosamine and chondroitin for the control of osteoarthritis in dogs, although this conclusion appears to be based solely on the Moreau *et al*. (2003) trial. Finally, the Comblain *et al*. (2016) commentary objectively presents the negative results of the Moreau *et al*. (2003) trial in contrast to the relatively positive results of the McCarthy *et al*. (2007), D'Altilio *et al*. (2007), and Gupta *et al*. (2012) trials without offering any conclusions or recommendations.

Discussion

Nutraceuticals are not considered medicinal products and are consequently not regulated by the United States Food and Drug Administration (FDA); therefore manufacturers are not required to provide scientific information to legal authorities for approval (Vandeweerd *et al*., 2012).

Health Canada, through the Veterinary Drugs Directorate (VDD) has the mandate to set standards for, evaluate and monitor the safety, quality, and effectiveness of, and promote the prudent use of veterinary drugs including veterinary natural health products (Health Canada, 2013). Despite the trend towards more stringent criteria for veterinary nutraceutical products, the research community continues to conduct and publish novel, low-quality studies without consistent evaluation methods and varying products/doses.

The lack of high-quality, peer-reviewed literature makes it difficult to draw conclusions about therapies. Nutraceutical studies commonly have limitations related to methods of participant recruitment and randomization, baseline characteristic data reporting, intervention standardization and concealment, blinding, participant retention, follow-up procedures, and overall protocol. In contrast, background, objectives, interventions, and statistical results tend to be well-reported (Vandeweerd *et al*., 2012).

Based on the available literature, the potential benefits of glucosamine and chondroitin use in osteoarthritic canines can neither be confirmed nor denied. Glucosamine and chondroitin use in canines requires further clinical study using improved methodology. Clinical trials conducted to date have yielded mixed results. These results are of questionable validity due to several trial shortcomings. First is the absence of therapeutic standardization. The sources of active ingredients, manufacturers of products, formulations, combinations of active ingredients, treatment doses, regimens, and durations of therapy differed significantly between trials. Second, multiple potential sources of bias were present in the trials, including the lack of a standardized follow-up timeframe, unexplained loss to follow-up, flawed study protocols, and incomplete data sets. Moreover, all of the clinical trials relied on subjective outcome measures to some extent, and the absence of standardized owner and veterinarian assessments increased the risk of bias in the reported results and diminished internal study validity.

The trials generally lacked peer review and were at risk of funding bias due to company sponsorship. Finally, there was an overall lack of generalizability of trial results. The trials were small in terms of the number of subjects used and subject baseline characteristics were not always disclosed. We cannot confidently extrapolate results from *in-vitro* studies using dogs with surgically/chemically-induced osteoarthritis to the client-owned dogs with naturally occurring osteoarthritis seen in practice.

Future study design proposal

From the above discussion, there is a clear need for a high-quality clinical study to evaluate the effect of glucosamine and chondroitin in canines with osteoarthritis. Table 3 proposes an ideal study design for a randomized clinical trial.

Our rationale for the study design aims to rectify common criticisms of previous study designs. We recommend conducting a multi-centered trial facilitated within veterinary orthopaedic surgery institutions and/or veterinary college institutions to eliminate funding bias. Client-owned dogs with naturally occurring osteoarthritis would be recruited and stratified according to disease severity (mild, moderate, or severe) using objective guideline measurements (i.e. radiographic imaging and semi-objective veterinary guideline assessment measurements).

Radiographic imaging to evaluate efficacy would include joint capsular distention, soft tissue thickening, and narrowed joint spaces. However, it is important to note that radiographic severity often does not correlate with clinical severity of disease. Thus, a standardized, semi-objective veterinary assessment would also be necessary for assessing disease severity and progression (Tilley and Smith, 2015). Once stratified, the dogs would be randomized into study arms using a central computerized system. All data collectors, analyzers, investigators, pet owners, subjects, and clinicians involved in the study would be blinded to the allocation of treatment.

Table 3. Future Study Design Proposal.

Patients	Client-owned dogs with naturally occurring osteoarthritis.
Inclusion	All breeds Age >1 year Weight >20 kg
Exclusion	Pregnancy; use of medications; hepatic, renal, or CV disease; gastrointestinal ulceration; bleeding disorder; lameness due to infectious, immune-mediated, neurological, or neoplastic disease.
Intervention	Dosages and regimen: 4 treatment arms 1) GHCl monotherapy GHCl 475mg BID for dogs 5-19.9 kg GHCl 712.5mg BID for dogs 20-40 kg GHCl 950mg for dogs >40 kg 2) GHCl and CS combination GHCl 475mg/CS 350mg BID for dogs 5-19.9 kg GHCl 712.5mg/ CS 525mg BID for dogs 20-40 kg GHCl 950mg/CS 700mg BID for dogs >40 kg 3) Crystalline glucosamine sulfate (unknown dose) 4) Placebo (control) Formulation: Liquid (appears to produce higher peak concentrations in comparison to tablets) (Maxwell et al., 2016). Administration: With food (typical home environments would have intervention administered in conjunction with food) (Maxwell et al., 2016).
Control	Placebo (liquid).
Open Label NSAID	Carprofen 2.2 mg/kg q12h.
Randomization	Stratified randomization based on disease severity.
Allocation Concealment	Central computerized random allocation, with all assessors, investigators, analyzers, owners, clinicians, and subjects blinded to treatment allocation.
Outcome	Primary outcomes: 1. Subjective: The owner's assessment of the pet's clinical presentation and quality of life using a standardized OA pain questionnaire (i.e. LOAD) 2. Semi-objective: A standardized clinical pain and OA assessment by a veterinarian 3. Objective: Radiographic changes, force plate gait analysis, static load bearing (to quantify reduced limb loading (Tilley and Smith, 2015)), and kinematics Secondary outcomes: Pharmacokintic characteristics of each dosage form, use of open-label NSAID, safety outcomes (adverse effects) and client/patient adherence.
Size	Calculated using the clinically significant difference in primary outcome score, expected standard deviation, and desired levels of confidence and power.
Baseline Characteristics Reported	Disease severity, number and location(s) of affected joints, weight, age, breed, athletic history, disorders that affect collagen or cartilage synthesis (Cushing's disease, diabetes mellitus, hypothyroidism) (Tilley and Smith, 2015).
Centre	Multi-centred, using veterinary or orthopaedic college institution(s).
Duration	≥90 days (potentially 1 year of follow-up if funding permits).

(CS): Chondroitin sulfate; (GHCl): Glucosamine hydrochloride; (LOAD): Liverpool Osteoarthritis in Dogs; (OA): osteoarthritis.

Baseline characteristics including age, number of affected joints, location of affected joints, breed, weight, comorbidities, and other medications used would be included in the study. Appropriate inclusion and exclusion criteria would be pre-specified. The study size would need to be sufficiently large to ensure internal validity through statistical adjudication.

The intervention in our proposed study would ideally be a multi-arm trial. A prospective superiority trial would consist of 4 treatment arms: 1) glucosamine HCl monotherapy, 2) glucosamine HCl and chondroitin sulfate in combination, 3) crystalline glucosamine sulfate and 4) placebo. Glucosamine and chondroitin are slow-acting agents; therefore, a study examining their long-term use would be appropriate. Serial blood samples would be helpful to determine pharmacokinetic characteristics of the different dosage forms. We would recommend a trial of at least 90 days in duration, with possible extension to 1 year of follow-up. However, longer treatment duration is often limited by cost and client/patient adherence to medication regimen. Conditions of treatment administration could

be further defined once efficacy has been established. A method of measuring compliance such as a client-completed dosing journal would be superior to reviewing the product returned by the owner, as dose absence does not necessarily equate to the successful administration of the dose. Additionally, analysing the data using both intention-to-treat and per-protocol analyses would establish the robustness of the results and reduce bias. For ethical reasons, allowing the open-label use of a standardized NSAID regimen (carprofen or meloxicam) for all subjects as needed would be ethically appropriate. NSAID use would be documented for both study arms and could be reported as a secondary outcome. The risks of all the interventions would be presented and explained to each dog owner using informed consent.

It is important to note that when drawing conclusions from a study, statistically significant results are not always indicative of clinical importance (Addleman, 2010). Pre-defining clinically meaningful results prior to the trial would help to establish whether or not the glucosamine and chondroitin intervention would be advantageous for use. Minimizing the risk of type 1 or type 2 errors by conducting a proper sample size calculation is also essential for producing trustworthy study results.

Objective guidelines and measures would be used to assess the baseline severity of osteoarthritis, clinical disease progression, and benefits of therapy. Primary outcomes would also be measured with a semi-objective standardized clinical assessment conducted by veterinarians or orthopedic surgeons. A thorough history, physical examination, and standardized pain-rating scale as well as objective measures such as radiograph, force plate gait analysis, and kinematic results would all be essential for generating a complete picture of the canines in the trial.

Additionally, standardized pain and activity scoring tools completed by the dog owners would provide subjective data as an adjunct to the objective and semi-objective data. The Liverpool Osteoarthritis in Dogs' Clinical Metrology Instrument (LOAD) is a good option for gathering subjective data from the dog owners as it is easy to use, validated, and has demonstrated a correlation with force-platform data (Walton *et al*., 2013).

The LOAD tool considers the pet's background, lifestyle, and mobility; specifically, it assesses the pet's level of exercise, activity, lameness, and stiffness, as well as the effects of the weather. As mentioned earlier, secondary outcomes to measure may include the frequency of open-label NSAID use.

Future studies require greater transparency to promote educated recommendations by veterinarians and pharmacists to dog owners. Studies should always disclose complete information with regard to the ingredients present in an intervention, subject baseline data, dates of recruitment and follow up, references of publications used, data for every item measured, and data analysis methods used such that readers can interpret the results independently.

Studies should also report the flux of participants using a flow chart that specifies the number of patients that were eligible, excluded, included, stratified, randomized, treated as intended, analyzed for the primary outcome(s), and lost to follow-up (Vandeweerd *et al*., 2012). Reporting the number of patients lost to follow-up and documented reasons for drop-out is standard safety reporting for clinical trials. Authors should determine the risk versus benefit ratios objectively. Lastly, the authors' conclusions and interpretations should be consistent with the study results.

Once higher-quality randomized controlled trials have been conducted, the quality of systematic reviews would also increase. Additionally, well-structured observational studies would help limit the heterogeneity of the results and ensure that fair comparisons between studies are made (Addleman, 2010). Literature should also be peer-reviewed to make the interpretation of study results and their application to practice easier.

Conclusion

Glucosamine and chondroitin are commonly recommended by veterinarians as an alternative for treating osteoarthritis in canines unable to tolerate the adverse effects of NSAIDs, or as add-on therapy. Although glucosamine and chondroitin have benign adverse effect profiles, the clinical benefit of using these agents remains questionable. The available evidence is difficult to interpret due to the use of different manufacturers, salt forms, compositions, sources, strengths, regimens, therapy durations, and combinations of active ingredients. Further study is required in order to clarify the uncertainty around the clinical benefit of using these agents and quantify any treatment effect that exists.

Conflict of interest statement

The authors declare that there are no conflicts of interest regarding the writing of this paper.

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