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WILL THE REAL HYALURONAN PLEASE STAND UP?

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This review intends to inform readers about the identity of Hyaluronan (Hyaluronic Acid or HA) materials currently used in dietary supplement products. As contestants from the TV game show "To Tell The Truth" (1956-1978) were made to choose the real person from two other foils by asking questions of each, this review will answer questions so that the real HA can stand up. There are major differences between the three major types of HA materials used in dietary supplements. According to the voluminous scientific literature, these differences may lead to distinctly different biological effects. This review does not attempt to discuss the rationale for oral HA as opposed to injectable or topical uses – this is an obvious topic for further review. However, ingestible HA products are available to the general public, and their use is growing to the point that HA-containing products are available from almost every mass-market retail provider. Consumers and health care professionals alike are not given facts about the identity of HA materials used in dietary supplements. Informed choices for evaluation of HA-containing products cannot be made without an understanding of what materials are used for each product.

HA PRIMER

HA is an extremely large molecule (normally 1-5 million daltons) of repeating subunits of N-acetylglucosamine and glucuronate (1-14). HA is classified as a glycosaminoglycan (GAG), and is present physiologically and commercially as the sodium salt (sodium hyaluronate). Single HA molecules exist as a hydrophilic, coiled mass approximately 200 nm in diameter, but form a mesh under typical *in vivo* conditions (8). HA is found everywhere in the body, but has especially important roles in joints. HA is the primary agent responsible for the viscosity and lubricating properties of synovial fluid (6, 8, 11-29). In cartilage, HA serves as the backbone of aggrecan proteoglycans, bound to core and link proteins (18, 30-49). Furthermore, HA is an important and under-recognized regulator of cell and tissue functions, as shown by the presence of specific receptors on all cells for HA and its fragments (8, 11-14, 50-65).

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The scientific and medical literature on HA and its effects on joints has had virtually no references to oral use of HA until very recently. Instead, HA has a prodigious literature on biological effects (6, 8, 11-14, 19-26, 28, 62, 66, 67), including a rather large number of *in vivo* animal and human clinical studies using intraarticular injections to treat osteoarthritis (OA) with purified HA (reviewed in 21, 22, 25, 26, 68-86). Thus, there is substantial knowledge of the biological effects of purified, high molecular weight HA on joint health.

HYALURONAN AS AN ORAL DIETARY SUPPLEMENT

HA as an oral supplement entered mainstream consciousness after an ABC News pro-

gram aired a segment that attributed possible anti-aging effects to dietary HA in November 2002. Since then, HA has been appearing as an ingredient in an ever-increasing number of dietary supplement products. HA-containing products have focused on joint and skin health. At the beginning of 2005, several of the largest dietary supplement companies were selling products containing HA in major retail outlets throughout the United States. This is in addition to other products available through Internet sales. In short, HA has catapulted from obscurity to mainstream store shelves rapidly.

There are a few animal studies on joint health after intravenous or intraperitoneal administration of HA (87-89), which provide some insight into tissue uptake and possible effects of HA after its appearance in the bloodstream. Four recent reports investigated effects of oral HA on animal musculoskeletal tissues. One report from the Hyaluronan 2003 Proceedings by Matrix Biology Institute administered 100 mg HA orally to 12 racing thoroughbreds for 59 days (90). Horses given HA were examined for lameness less frequently than 13 control group horses, suggesting that oral HA prevented lameness in active horses. Another report by Stancikova *et al* administered HA (0.5-1 mg/kg) with molecular weights of 0.75 and 1.62 million daltons by oral routes for eight weeks to ovariectomized rats (91). Both sizes of HA showed increased serum concentrations of nitric oxide (NO), but only the larger HA showed decreased markers of bone resorption as well as an inhibition of bone mineral density loss. Two other animal studies were found on the Contipro website (92). Oral HA fed to rats at a dose of 0.1 mg/kg showed reduced swelling of hind leg and decreased NO production after arthritis induction by Freund's adjuvant. An open study from 7 veterinary clinics studied oral HA given to 53 racehorses with inflammatory and degenerative joint conditions. After 30 days of oral HA, symptoms were reduced and functionality improved. All reports used purified, high-molecular weight HA from a microbial fermentation source (Nutrihyl®, Contipro Group Holding, Czech Republic).

There are unpublished reports of oral HA in humans for joint health from the Czech Republic. A product named Chondrorevit was reported to improve joint health in subjects with knee OA or after knee surgeries (92). Daily doses of 20 mg HA and 400 mg chondroitin sulfate were administered for 90 days. Improvements in joint function or recovery time after surgery were reported. Interestingly, this amount of chondroitin sulfate has not been shown to have significant efficacy in dose-finding studies from Europe (93), and thus, any benefits may be attributable to the HA or the combination of HA and chondroitin at a less than efficacious dose. However, these studies did not appear to be randomized, double blind or placebo controlled, and thus, their findings must be verified in controlled studies before results can be relied upon. Nevertheless, HA has been reported to have benefits for joint health after oral use in animal and human clinical studies. At this point in time, reports of oral HA for joint health have all used high molecular weight, purified HA, except for one study discussed later in this review.

MOLECULAR WEIGHT AND BIOLOGICAL EFFECTS OF HYALURONAN: EVIDENCE SHOWING THAT SMALL HYALURONAN AND FRAGMENTS ARE DIFFERENT

Because HA is a repeating polymer devoid of modifications (such as branching or sulfation), major differences in biological activities for HA are attributable to its molecular weight (size) (25, 65, 67, 94-98). There are clear cutoffs of molecular weight for lubricating properties (17, 19, 25, 99-106), cell signaling (107-117), and perhaps for efficacy of injectable intraarticular preparations for OA treatment (63, 67, 118-120). Molecular weight of less than 500,000 daltons seems to be

the cutoff for differing properties of HA. This concept is summarized by a comment from Camenisch and McDonald: "The work of Ohkawara and coworkers and numerous other observations suggest that there is something fundamentally different about the biological response to high (megadaltons) and lower molecular weight HA." (95). In essence, bigger is better for health benefits from HA, and smaller fragments have very different or opposite properties than native, high molecular weight HA.

Very low molecular weight HA fragments cannot be expected to have the same biological effects as high molecular weight HA, as is clear from the growing literature on biological effects of HA. Very low molecular weight fragments either do not bind to specific cell membrane receptors that recognize native HA, or they transduce different signals to cells (65, 107, 108, 113, 121-126). For example, very low molecular weight HA fragments (6.9 kDa), but not high molecular weight HA, have been associated with promotion of cancer cell neovascularization, migration and metastasis (112, 117, 122, 127-132). Low molecular weight HA fragments have either lost anti-inflammatory effects or instigate proinflammatory, catabolic properties compared to native HA in several biological systems (64, 110, 114, 116, 121, 125, 132-141). For example, HA fragments of 250,000-dalton molecular weight induced expression of inflammatory genes in macrophages (64, 65, 132, 135, 142). These effects are not due to contamination, as shown by the fact that the size range of HA tested *in vitro* is the same range as is found *in vivo* during inflammation (143). Some reviewers have postulated that HA fragments promote and propagate OA (65, 97, 136, 140, 144). These are obviously not desirable traits for dietary supplements purporting to support joint health.

TABLE 1: Identity and Characteristics of Hyaluronan Materials Used in Dietary Supplements

Hyaluronan Type	Source	Molecular Weight (daltons)	Purity	References
Hydrolyzed chicken sternal cartilage Type II Collagen	Chicken sternal cartilage	50–10,000[1]	~10%	• Ishaq S. Hyaluronic acid and chondroitin sulfate based hydrolyzed collagen type II and method of making same. United States Patent 6,780,841, August 24, 2004. • http://www.biocell technology.com
Rooster Comb (Injuv™)	Chicken comb	50,000–200,000	9%	• Udell RG, Naguib YMA. Hylaluronic acid in soft gel form. United States Patent 6,806,259, October 19, 2004. • http://www.injuv.us
Sodium hyaluronate	Microbial fermentation (Streptococcus species) or chicken comb	700,000– 1 million or higher	90+%	• http://www.bioiberica.com/jointcare/hyaljoint.htm • http://www.cpn-contipro.com

[1]Patent 6,780,841 (column 6, line 12) stated: "The average molecular weight of the final product is between 50 and 10,000 daltons, preferably 5,500 daltons." Neither the patents nor website specifically list a range of molecular weight for hyaluronan itself. Methods of analysis to determine molecular weight or identity of HA were not stated.

HA MATERIALS

There are three major types of commercially available HA materials used in dietary supplements. Table 1 lists these types and their basic attributes. Information was obtained in the public domain from material supplier or supplement company websites. It is readily apparent that there are major differences in molecular weight of each type, yet all are labeled identically as Hyaluronic Acid or Sodium Hyaluronate on dietary supplement product labels (see Table 2). Only one type of HA matches what is normally present in the human body – high molecular weight, purified (protein-free) HA. The other two major types of HA materials have lower molecular weights.

HYDROLYZED CARTILAGE HA (BIOCELL COLLAGEN II™)

HA from hydrolyzed cartilage has a patent describing the enzymatic degradation of Type II collagen from chicken sternal cartilage into very small fragments (50 to 10,000 daltons), with no high molecular weight material remaining (145). This process results in a material consisting of 10% Hyaluronic Acid, 20% depolymerized chondroitin sulfate, and around 63% Type II collagen fragments (145). This review is not concerned with the collagen or chondroitin contents of this material, which may have their own effects on joint health. However, the HA in this material is pertinent since it is used in dietary supplement products listed as a source of HA (see Table 2). It is not stated in the patent or in company website information what size range of HA fragments exist in this material. The patent further states (column 7, line 46):

> "By extracting HA from chicken sternal cartilage, a low molecular weight HA may be obtained by hydrolysis after such extraction. This distinction is crucial because the beneficial therapeutic activity of HA is mostly dependent upon the molecular weight of HA. Due to the low molecular weight of the HA found in the hydrolyzed collagen type II, the hydrolyzed collagen type II readily absorbs into the gastrointestinal tract and allows the rejuvenating constituents of HA and CSA to restore viscoelasticity to the skin, protect connective tissues, promote cartilage synthesis, retain skin moisture, heal wounds and improve the overall appearance of skin."

The patent does not supply any data on the size of HA, the analytical method used to assess HA identity, or any effects of the material. In other words, there is no supportive data to substantiate the properties attributed to HA in the statement above. However, it is clear that the inventor assumes that the HA in this material is of low molecular weight.

As briefly discussed previously in this review, HA fragments do not have the same properties in biological systems as native, high-molecular weight HA does, and thus, this material does not fit the definitions and descriptions of HA agreed upon by textbooks, reviewers and experts in the field (8, 11-14, 19, 23-26, 28, 62, 113, 125, 126). In short, this material is not typical HA and thus should not be described as HA on dietary supplement labels. Rather, this material is more accurately described as very low molecular weight HA oligosaccharides or even predigested HA. This statement assumes that the HA in this material is indeed hydrolyzed, which at present is not substantiated.

Furthermore, HA in cartilage does not exist as free, unbound molecules, but rather, as the backbone of aggrecan proteoglycans (18, 30-49). As such, HA is bound to link and core proteins, and sometimes to other GAGs or chondrocytes themselves. Even assuming proteolytic enzyme treatment as described in the patents to prepare this material can cleave HA from protein (145-147), the presence of amino acids crosslinked to HA fragments are not synonymous with a definition of HA.

TABLE 2. Examples of How Dietary Supplement Products Containing Hyaluronan Are Labeled[1]

Product	Description of Hyaluronan in Supplement Facts	Amount per serving	Accompanying Label Claims
21st Century Healthcare, Inc. Arthri-Flex	Hyaluronic Acid	Not stated—part of a Flexicol™) proprietary blend with four other ingredients totaling 645 mg	• With Flexicol Premium Chicken Sternum Type II Collagen • Containing: Premium Chicken Sternum Cartilage Type II Collagen (naturally occurring Chondroitin Sulfate and Hyaluronic Acid) • Contains Highly Absorbable Hyaluronic Acid • www.21stcenturyvitamins.com
Arthritis Research Corp. Flex-a-min® Complete™	Hyaluronic Acid (as Sodium Hyaluronate)	Not stated—part of a proprietary blend with three other ingredients totaling 400 mg	• Hyaluronic Acid—The Glucosamine in Flex-a-min® Complete™ promotes the production of Hyaluronic Acid in the body, including the synovial fluid surrounding joints. • www.flexamin.com
Arthritis Research Corp. Flex-a-min® Triple Strength	Hyaluronic Acid (as Sodium Hyaluronate)	Not stated—part of proprietary blend with Silica totaling 40 mg	• Plus, we added the popular ingredient Hyaluronic Acid to set this formula apart. • Hyaluronic Acid—The Glucosamine in Flex-a-min® Complete™ promotes the production of Hyaluronic Acid in the body, including the synovial fluid surrounding joints. • www.flexamin.com
Injuv™ Hyaluronic Acid	Hyaluronic Acid	12.6 mg (from 140 mg Injuv™)[2]	• Stop the stiffness, stop the pain and increase your mobility with Injuv™ (www.jointlubricant.com/injuvworks.htm) • The HA in Injuv™ stops pain and inflammation of joints, because it helps to hydrate and lubricate the synovial cell membrane. (www.jointlubricant.com/injuvworks.htm) • Injuv™ has an exclusive patent on the process that breaks down HA so that it can be taken orally. • The hyaluronic acid molecule in Injuv has been enzymatically cleaved into smaller fragments. (www.injuv.us)
Nature's Bounty Hyaluronic Acid	Hyaluronic Acid (as Sodium Hyaluronate)	20 mg per capsule	• Hyaluronic Acid is found in almost all adult connective tissue. Hyaluronic acid is a natural component of the body. • www.NaturesBounty.com
Schiff® Move Free Triple Strength	Hyaluronic Acid (in Other ingredients list)	Not stated (Consumer Service line stated 3.3 mg per serving)	· Now with Joint Fluid (Hyaluronic Acid) • www.schiffvitamins.com
Product			

TABLE 2. Continued

Product	Description of Hyaluronan in Supplement Facts	Amount per serving	Accompanying Label Claims
Spring Valley Glucosamine & Collagen with Chondroitin & HA (Hyaluronic Acid)	Hyaluronic Acid	40 mg	• In a base of amino acids (Hydrolyzed Collagen Type II) • Collagen Type II 500 mg • Chondroitin Sulfate (Avian, Porcine, Bovine) 100 mg • Collagen is the most plentiful protein and building block in the body and is a natural source of Chondroitin, a nutrient shown to regenerate joint tissue, and Hyaluronic Acid (HA), a revolutionary compound that retains moisture and functions as a lubricant between connective tissues.* • Hyaluronic acid is fast absorbing in the blood and thus available for joint tissues. • Lot number 4MA0236
Swanson Ultra® Hyal-Joint™	Hyal-Joint™ Hyaluronic Acid	20 or 50 mg per capsule.	• The unique hyaluronic acid formulation in Hyal-Joint has the proven ability to be absorbed in the intestinal tract and has demonstrated significant joint-protective properties in numerous studies. • www.bioiberica.com
Windmill™ Health Products Glucoflex™ Hyaluronic Acid	Hyaluronic acid sodium salt	10 mg per tablet	• Advanced Joint Lubricating Formula with Glucosamine • Glucoflex™ Hyaluronic Acid formula with Glucosamine supplies the most bioavailable form of HA (Hyaluronic Acid) for the most direct delivery of this amazing nutrient.

[1]Products were obtained from store shelves from the United States or from internet website information. Products are listed in alphabetical order according to manufacturer name.
[2]Some products containing Injuv™ HA list the total amount of material instead of the actual amount of HA, misleading consumers into believing there is 70 or 140 mg of HA per softgel.

Another issue apparently not considered by some marketers of hydrolyzed cartilage HA is the integrity of borrowing literature describing properties of HA to support such product claims (see Table 2). Since hydrolyzed HA has quite different physical and biological properties compared to high molecular weight HA, it is a different entity. For products with very low molecular weight HA (hydrolyzed cartilage) it is inappropriate to make claims, such as lubricating properties, which rely on literature describing high molecular weight HA. This is especially true when there is also evidence of very low molecular weight HA not having the properties claimed (such as lubricating properties). Thus, it would be more appropriate to base any claims for joint health with this material on studies using this material.

Recently, a late-breaking abstract presented at the 2004 FASEB meeting in Washington DC described the results of a human clinical study using hydrolyzed cartilage material (148). A press

release from BioCell Technology, LLC posted on their website on April 2003 also reiterated the results of the abstract (149). In the abstract, the material was described as ". . . a hydrolyzed type II collagen product (avian source: Biocell™; HC2)" without mention of HA. A dose of 2000 mg daily was given to eight subjects with knee or hand osteoarthritis for two months, and compared to a placebo group of eight subjects in a randomized, double blind, placebo-controlled study. WOMAC scores were improved significantly greater in the HC2 group compared to placebo, but no data were presented in the abstract or press release. This amount of material would contain approximately 200 mg of hydrolyzed HA daily, according to the supplier's website and patent. However, approximately 1600 mg of hydrolyzed Type II collagen and 400 mg of chondroitin sulfate fragments were also present in this material. Thus, the contribution of HA to observed results is unclear. Furthermore, human studies with subjective questionnaire endpoints (WOMAC) and with only eight subjects per group run a high risk of low statistical power, making results preliminary until confirmed in more powerful studies. Most other studies that mixed subjects with knee and hand osteoarthritis have been criticized since the arthritic process in hand osteoarthritis is distinctly different than in knees.

In addition, a press release by BioCell Technology from February 2, 2004 described a 36-hour peak absorption study conducted in an unknown number of human subjects with hydrolyzed cartilage material (150). HA levels were determined after a single dose and after 28 days of administration. Levels of HA in blood were claimed to be 7008.62% above control levels after 12 hours. Also, two HA metabolites 1/600th the size of the ingested HA were found. After 28 days, HA levels in blood were reported to be 3542.58% above control, and metabolites at 11,890.15% above control. No details on blood collection or analytical methods were given, although the reporting of HA metabolites with two decimal places implies a high degree of precision for the method of analysis. Normal human serum levels of HA are 0.01-0.1 mg/L (mcg/ml) (8). Assuming the press release was describing serum HA levels, a 7000% increase in blood HA levels would produce levels of 0.7-7 mcg/ml, which is within the range seen for certain cancers, liver conditions and inflammatory diseases (8). One possible interpretation of this study is that this material elevates serum levels of HA fragments, along with native HA levels, although more experimental details are needed to confirm this interpretation because the effects of activity and food intake are known to increase plasma HA levels (151). Absorption of HA from the material is not proven by these results, since HA can rapidly enter circulation, especially in inflammatory diseases (8). Thus, another interpretation of these results is that this material is pro-inflammatory, which in and of itself would elevate serum levels of HA, congruent with findings of other researchers on pro-inflammatory effects of very low molecular weight HA fragments.

Assay of HA fragments, especially in a mixture with chondroitin sulfate or its hydrolysis products, is problematic. In fact, assay of products containing very low molecular weight HA fragments by validated assays utilizing HA binding protein technology do not find any measurable HA (Bucci, L., unpublished results). This indicates that if present, HA fragments are less than eight subunits in length. These assays also indicate that there is no high molecular weight HA present, so claims for this material on product labels or websites based on properties of high molecular weight HA cannot be substantiated by borrowing from the literature describing high molecular weight HA.

Therefore, HA derived from hydrolyzed chicken sternal cartilage is characterized by extremely low molecular weights, a lack of identity as native HA itself, a probable difference in biological effects (assuming uptake and distribution to tissues), a lack of transparency on how

the HA content was verified, and inability to show presence of HA in validated assays for HA using specific HA binding protein methodology. In addition, some products do not describe this material as hydrolyzed (see Table 2), which misleads consumers into thinking that they are getting native HA. Consumers expect to receive high molecular weight HA since this is what media, medical usage and website information all describe as characteristic of HA. This material is easy to identify in products because of the presence of Type II Collagen, chicken (avian) sternal cartilage source, and simultaneous presence of HA, chondroitin sulfate and Type II collagen naturally. These facts argue strongly that this material should not be labeled as HA in dietary supplement products as the identity and putative biological effects are much different from that of high molecular weight HA.

ROOSTER COMB HA (INJUV™)

The second major type of HA used in dietary supplement products is prepared from dried rooster comb, a rich natural source of HA (7500 mg/L) (8). This material is claimed to be enzymatically denatured, with a molecular weight of 50,000 to 200,000 daltons in order to facilitate uptake, without any supporting evidence (152). Injuv™ HA is identified by validated HA binding protein assays (Bucci L, unpublished data). However, experts in the field regard HA with a molecular weight of 50,000-200,000 daltons to be low molecular weight HA. Thus, HA in this size range has lost its ability to form networks in solution, and thus, has lost viscosity and lubricating properties.

Inspection of HA in intact rooster combs shows tight association of HA with proteins (153-155). It is unclear if conditions used to prepare rooster comb HA with enzymatic hydrolysis would actually dissociate the tightly bound proteins from the HA, since details of preparation are lacking in the published patent. High molecular weight HA prepared from rooster combs for injectable use, such as Hyalgan® Sodium Hyaluronate from Sanofi-Synthelabo Inc., has been extensively treated to remove proteins in order to prevent reactions after injection, and has not shown appreciable adverse effects after intraarticular injections (156). Such material would fit into the third type of HA material.

HA with molecular weights of less than 500,000 daltons exhibit decreased viscosity and lubricating properties in solution, as stated earlier in this review. HA in this size range has lost its ability to form networks in solution. Therefore, claims about lubricating properties are inappropriate with this material unless specific studies are performed to investigate its lubricating effects in joints. Also, signals transduced by HA in the molecular weight range of 100,000-200,000 are different from those of HA at 1 million daltons, and generally are proinflammatory. The presence of tightly bound proteins in rooster comb HA has uncertain implications for uptake of this type of HA. Digestion and uptake of rooster comb HA may be different from native, purified HA. So this material, while clearly low molecular weight HA, is different from native, high molecular weight HA and has been shown to have different biological effects.

PURIFIED HA

The third major type of HA available for dietary supplement products is essentially purified HA (Table 1). Typical commercial sources are from microbial fermentation (*Streptococcus* species) or rooster combs. Molecular weight is around 1 million daltons, which corresponds to native HA in cartilage and in synovial fluid. Protein is absent or very low in these preparations. HA from microbial fermentation sources can be declared as vegetarian, whereas HA derived from any cartilage or chicken comb sources cannot. Since purified HA for food use is produced by manufacturers that also produce purified HA for pharmaceutical use, there is more certainty that these products have a tradition of quality

TABLE 3. Biological Properties of High Molecular Weight Hyaluronan and Hyaluronan Fragments

Molecular Weight (daltons)	Biological Property	Reference
Very low molecular weight oligosaccharides (800-5000)	• Angiogenic—Induced angiogenesis in rat skin grafts, healing wounds, chick chorioallantoic membranes • Angiogenic—Induced rapid transient up-regulation of the immediate early genes c-fos, c-jun, jun-B, Krox-20 and Krox-24 that control angiogenesis • Angiogenic—Increased tyrosine kinase, protein kinase C cascades in endothelial cells • Angiogenic—Induced proliferation of bovine brain and aortic endothelial cells • Antiinflammatory—Tetrasaccharides, but not larger sizes of HA, induced heat shock protein 72 in K562 cells and prevented cell death of PC12 cells after serum deprivation. • Antioxidant—Exhibited antioxidant activity against superoxide, but less than higher molecular weight HA; did not scavenge hydroxyl radicals (unlike high molecular weight HA) • Catabolic—Did not affect sulfate incorporation in chondrocyte cultures • Catabolic—Blocked binding of aggrecan HA to chondrocytes, resulting in loss of staining for proteoglycans (disrupted cartilage structure) • Catabolic—Caused chondrolysis (loss of chondrocytes) when hexasaccharides exposed to cartilage tissue slices and chondrocyte cultures • Inflammatory—High molecular weight HA cleaved at sites of inflammation into small fragments • Inflammatory—Activated macrophages and dendritic cells in healing wounds • Inflammatory—Induced maturation of dendritic cells to tissue macrophages • Inflammatory—Induced production of IL-1beta, TNF-alpha, IL-12 and iNOS in dendritic cells and macrophages • Inflammatory—Did not inhibit arachidonic acid release after bradykinin from synovial fibroblasts from osteoarthritic subjects • Inflammatory—Did not inhibit MMP-1 and RANTES expression or production in normal and osteoarthritic human chondrocytes in culture • Inflammatory—Induced expression of matrix metalloproteinases, but not TIMPs, in normal rabbit and bovine cartilage chondrocytes • Inflammatory—Induced MMP-9, MMP-13 expression and production in murine fibroblasts and 3LL cells • Inflammatory—Stimulated MAP kinase and urokinase-type plasminogen activator in human HCS-2/8 chondrosarcoma cells • Inflammatory—Did not inhibit neutrophil adhesion or aggregation under conditions of shear and turbulent flow • Inflammatory—Stimulated tyrosine phosphorylation and c-Met expression in human chondrosarcoma cells	7, 8, 11, 15, 20, 28-32, 35, 38, 39, 42, 46, 52, 53, 57, 59, 60-63, 67-69, 71-73

TABLE 3. Biological Properties of High Molecular Weight Hyaluronan and Hyaluronan Fragments (*continued*)

Molecular Weight (daltons)	Biological Property	Reference
24,000	• Catabolic—Inhibited tumor growth, induced apoptosis • Inflammatory–Induced expression and production of inflammatory mediators from murine macrophages (Il-1, TNF-alpha, IL-12, iNOS, metalloelastase) • Lubrication—Reduced elasticity & viscosity of 1.3MDa HA solutions (competed for meshwork formation)	13, 23, 36, 37, 43, 44, 54
33,000	• Antiangiogenic—Did not induce angiogenesis of rat skin grafts	35
<50,000	• Catabolic—Did not stimulate HA synthesis or inhibited synthesis (high concentrations) of HA from human osteoarthritic synovial fibroblasts • Inflammatory—Did not inhibit proliferation of human mononuclear cells	47, 58
50,000	• Inflammatory—Did not inhibit MMP-1 and RANTES expression or production in normal and osteoarthritic human chondrocytes in culture • Inflammatory -—Induced expression and production of inflammatory mediators from murine macrophages (Il-1, TNF-alpha, IL-12, iNOS, metalloelastase)	23, 36, 37, 43, 44, 61
90,000	• Catabolic—did not enhance bone mineralization in rat calvarial cells • Inflammatory—Did not inhibit phagocytosis by mouse peritoneal macrophages at same viscosity as higher molecular weight HA • Lubrication—Osmotic pressure opposing fluid drainage in rabbit joints reduced, faciltates escape of fluid and HA from joints	6, 10, 24
80,000–200,000	• Antiinflammatory—Did not induce matrix metalloproteinase production in murine fibroblasts and 3LL cells • Antiinflammatory—Did not induce IL-1beta, TNF-alpha or IL-12 in human dendritic cells or macrophages • Antioxidant—Scavenged hydroxyl radicals in vitro, but was les effective than high molecular weight HA for protecting tendon fibroblasts from hydroxyl radicals • Inflammatory—Did not inhibit cell proliferation of rabbit synovial cells • Inflammatory—Induced expression and production of inflammatory mediators from murine macrophages (Il-1, TNF-alpha, IL-12, iNOS, metalloelastase)	8, 16, 23, 36, 37, 43, 44, 49, 62, 63
200,000	• Inflammatory—Markedly stimulated iNOS mRNA in liver endothelial and Kuppfer cell types • Inflammatory—Intravenous HA (250,000 daltons) did not inhibit proinflammatory cytokine levels in serum after liver injury in rats • Inflammatory—In human eosinophils, increased production of intercellular adhesion molecule-1, TGF-beta, protein secretion, and transformedcells from round to spindle shapes	19, 40, 45, 51

TABLE 3. Biological Properties of High Molecular Weight Hyaluronan and Hyaluronan Fragments (*continued*)

Molecular Weight (daltons)	Biological Property	Reference
	• Inflammatory—Human peritoneal mesothelial cells produced more inflammatory cytokines (MCP-1, IL-8)	
280,000	• Anticatabolic—Inhibited cartilage degradation by neutrophils, but significantly less than 950,000 or 2.0 million dalton HA • Inflammatory—Produced inflammatory cytokines RANTES, IL-12, MIP from murine macrophages	21, 65
350,000	• Lubrication—Formed poor meshworks with reduced viscosity • Lubrication—Osmotic pressure opposing fluid drainage in rabbit joints reduced, facilitates escape of fluid and HA from joints	6, 54
460,000–2.8 million	• Antiinflammatroy—Dose-dependent inhibition in phagocytosis from mouse peritoneal macrophages (steric hindrance) • Antiinflammatory—Did not induce matrix metalloproteinases in normal rabbit and bovine cartilage chondrocytes • Antinociceptive—Molecular weight-dependent improvement in abnormal gait in rats acute arthritis, indicating nociceptive effects with higher molecular weight HA	1, 10, 17, 46
Native HA (approx. 800,000 to 6 million)	• Anabolic—Stimulated synthesis of high molecular weight HA from human osteoarthritic synovial fibroblasts • Anabolic—Increased proteoglycan synthesis from IL-1Beta treated chondrocytes • Anabolic—Stimulated fibroblast proliferation in a collagen lattice, but not in fibroblast monolayer • Anabolic—Enhanced proteoglycan synthesis in human cartilage explants after fibronectin fragment treatment • Anabolic—Enhanced chondrocyte proliferation and chondroitin sulfate synthesis in rabbit chondrocytes embedded in collagen gels • Anabolic—Forms pericellular matrix (with aggrecan) in presence of chondrocytes • Anabolic—Improved bone mineralization in rat calvarial cells • Antiangiogenic—Inhibited stimulation of angiogenesis and early response genes by low molecular weight HA fragments • Antiangiogenic—Inhibited or did not induce angiogenesis of chick chorioallantoic membranes or endothelial cells • Antiangiogenic—Did not cause proliferation of bovine brain and aortic endothelial cells • Anticatabolic—Decreased reduction in proteoglycan synthesis from fibronectin fragments • Anticatabolic—Decreased cartilage degradation from fibronectin fragments • Anticatabolic—Decreased expression of matrix metalloproteinase 3 in human cartilage explants after fibronectin fragment treatment • Anticatabolic—Inhibited leukocyte elastase and lysozyme release • Anticatabolic—Inhibited neutrophil-mediated cartilage degradation (reduced release of sulfated GAGs from bovine nasal cartilage explants) • Antiinflammatory—Did not induce iNOS mRNA from liver cell types	1-8, 10, 12, 15-18, 20-22, 24-29, 32-34, 38, 39-44, 45, 47-51, 53, 58, 59-63, 66, 68, 70-72, 74, 75

TABLE 3. Biological Properties of High Molecular Weight Hyaluronan and Hyaluronan Fragments (*continued*)

Molecular Weight (daltons)	Biological Property	Reference
	• Antiinflammatory—Reduced IL-1beta, TNF levels in rat air pouches • Antiinflammatory—Did not induce IL-1beta, TNF-alpha or IL-12 in human dendritic cells or macrophages • Antiinflammatory—Inhibited PGE2 production by human osteoarthritic synovial cells and normal rabbit articular chondrocytes after IL-1alpha induction • Antiinflammatory—Did not induce differentiation of human dendritic cells into macrophages • Antiinflammatory—Decreased mitogen-dependent activation of human mononuclear lymphocytes • Antiinflammatory—Inhibited proliferation of rabbit synovial cells in culture • Antiinflammatory—Inhibited carrageenin-induced edema in dose-dependent manner • Antiinflammatory—Inhibited adjuvant arthritis in dose-dependent manner • Antiinflammatory—Inhibited arachidonic acid release induced by bradykinin in synovial fibroblasts of osteoarthritic humans • Antiiflammatory—Inhibited advanced glycation endproducts from activating NF-kappaB, IL-1alpha, IL-6, TNF-alpha • Antiinflammatory—Inhibited MMP-1, RANTES expression and production in normal and osteoarthritic human chondrocyte cultures • Antiinflammatory—Did not enhance MAP kinase, urokinase-type plasminogen activator, tyrosine phosphorylation or c-Met in human HSC-2/8 chondrosarcoma cells • Antiinflammatory—Enhanced production of TIMP-1 in normal bovine chondrocyte culture, lowered stromelysin/TIMP-1 ratios • Antiinflammatory—Decreased synovial PGE2 and bradykinin in arthritic rats after intraarticular injection • Antiinflammatory—Inhibited neutrophil aggregation and adhesion under conditions of shear and turbulent flow • Antiinflammatory—Intravenous HA (>780,000 daltons) decreased proinflammatory cytokines and liver enzymes after liver damage in rats • Antiinflammatory—Did not cause eosinophils to produce inflammatory cytokines • Antiinflammatory—Did not induce inflammatory cytokines RANTES, IL-12, MIP from murine macrophages • Antioxidant—Decreased free radical formation from IL-1Beta treated chondrocytes • Antioxidant—Scavenged superoxide and hydroxyl radicals dose-dependently • Antioxidant—Protected tendon fibroblasts in culture from damage by hydroxyl radicals • Lubrication—High molecular weight HA (>500,000 daltons) caused outflow buffering in rabbit joints, reducing drainage and loss of HA and fluid • Regulatory—Inhibited sulfate incorporation into chondrocyte cultures	

TABLE 3. *Continued*

Note: Filion and Phillips determined that some (2/7) of both high (0.5-2 million daltons) and low (10,000-300,000) molecular weight preparations of HA were inflammatory (produced IL-12 and TNF from human monocytes) due to contaminating DNA (based on reduction of activity after DNAse treatment). This suggested that a low percentage of individual HA preparations have potential to cause inflammatory events not due to the molecular weight of HA. However, many of the studies cited in Table 3 examined different molecular weights from the same source of HA, and noticed an effect dependent on molecular weight, indicating contamination was not involved. The large number of studies showing an effect dependent on molecular weight from a wide variety of investigators, preparations and biological sources also argues strongly that DNA contamination is not operative in at least a majority of studies, or else no differences dependent on molecular weight would have been found. Scott and Heatley provide tertiary structure evidence that smaller HA fragments are different from high molecular weight HA, helping to explain the differences in biological activities of denatured HA.

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control, toxicological testing and reproducibility than the other two types of HA material.

As mentioned previously in this review, HA with high molecular weight of 1 million daltons has viscosity and lubricating properties, and sends antiinflammatory, anabolic signals to cells. Indeed, one mechanism of action proposed for intraarticular HA treatments of OA is the normalization of cell signaling to the synovium from high molecular weight HA (66, 71). Table 3 lists some of the biological properties associated with high molecular weight HA as well as HA fragments. Products using high molecular weight HA match the identity of native HA in the body and from the scientific literature. Claims relating properties and actions of HA from products containing high molecular weight HA appropriately borrow evidence from the scientific literature. Thus, high molecular weight, purified, native HA is the most logical choice for use as HA material in dietary supplements.

ORAL ABSORPTION OF HA

Of course, one prerequisite for any beneficial effects from HA in dietary supplement products is uptake into bloodstream and joint tissues after oral intake. Of the three types of HA materials, only one type, purified HA, has published evidence of oral absorption. An abstract from the 2004 FASEB meeting in Washington DC described uptake of labeled HA into the bloodstream and localization of the label into the joints and salivary glands of rats and dogs several hours after oral administration (157). The HA in this study was derived from microbial fermentation, and was 1 million daltons. Since 99m-technetium was used as the label, and control experiments showed that the label was not removed or exchanged from HA molecules (Bucci, L., unpublished data), the presence of the label in the joints is most likely explained by the presence of the HA absorbed after oral

administration. This study is the first published report of oral absorption and tissue uptake of oral HA and provides the rationale for HA in dietary supplements.

Another source of purified HA from rooster comb has stated on the company website that absorption of their material has been found (158). However, no details or data have been presented or published. Hydrolyzed chicken sternal cartilage material is likewise claimed to have not only absorption, but superior absorption to native HA because of its smaller size. This claim is also on product labels or accompanying text (see table 2 and previous sections of this review). Again, website information from the manufacturer describes that absorption was found in a study, but no details or data have been presented or published in a scientific forum. No head-to-head comparisons to other HA materials concerning uptake have been reported, rendering claims of superior absorption unsubstantiated and unsupported. Only one type of HA—purified, high molecular weight HA from microbial fermentation—has published evidence for oral uptake and distribution to connective tissues.

BENEFICIAL PROPERTIES OF HA FOR JOINT HEALTH

Oral HA products are mostly targeted to joint and skin health, according to structure and function claims on product labels and accompanying literature (for a sampling, see Table 2). These two uses coincide with the prevalence of HA in the human body (8). Almost all beneficial properties for joint health attributable to HA have been documented for high molecular weight HA unbound to protein. Extensive *in vitro* studies using purified HA have uncovered beneficial properties for joints (see table 3). In addition, publication of a large number of human clinical studies using intraarticular injection of HA has demonstrated direct benefits for joint health (reviewed in 21, 22, 25, 26, 68-86). Of particular interest are the human studies showing repair or normalization of synovium and cartilage structure and architecture for long time periods after a course of injections to persons with OA (66, 71, 159-164). These changes were documented by before and after tissue biopsies, along with biochemical measurements long after the injected HA disappeared from joints. These studies show direct evidence in humans that presence of purified, high molecular weight HA in joints has long-term benefits months after exogenous HA was given. The results of the oral uptake study presented at FASEB (157) show that oral HA has the potential to reach joints, and provide a strong rationale for use of oral HA in dietary supplements. However, at this time, this rationale applies only to purified, high molecular weight HA from microbial fermentation sources.

SUMMARY

It is interesting to speculate that continued oral administration of HA might produce similar or superior effects to intermittent injectable HA, as has been seen for both glucosamine and chondroitin sulfate. However, dietary supplements containing HA are not equivalent due to the inherent properties of the three major types of HA commercially available as dietary supplement materials. Consumers and health care professionals need to be aware of the different types of HA and their very large differences in properties (even before ingestion). One source, hydrolyzed chicken sternal cartilage, is clearly unlike native HA, does not match the biological properties of native HA, and consequently should not be represented as HA to consumers on product labels. Rooster comb HA is significantly smaller than native HA (about 10-20% the size of native HA), and is tightly bound to connective tissue proteins. This type of HA does not match the literature on properties and benefits of native HA because of its smaller size and tight binding to proteins. Finally, high molecu-

lar weight, purified HA is available from vegetarian-compatible and animal sources, virtually identical to native HA and pharmaceutical preparations of HA. This material is analogous to the HA used to generate the extensive body of knowledge on HA, both its roles and its therapeutic effects in OA. HA from the microbial fermentation method of preparation has published evidence for uptake into joints after oral administration. From all considerations, purified, high molecular weight HA from microbial fermentation in dietary supplement products has the highest likelihood for matching the known attributes of HA. The real HA—purified, high molecular weight—can stand up now.

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