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Is Oral Nutritional Supplementation Truly Effective in Preserving Joint Health and Protecting Joints? A Bird's-eye view of the Consolidated Evidence and an Investigation of the Contribution of Nucleotides

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ABSTRACT

***Background**: Amateur sports practitioners in Europe and North America number roughly 400-450 million, a vast market for joint health and protection. The search for joint protection should also extend beyond sports-related injuries to the general population, especially aiming at osteoarthritis prevention. Glucosamine, chondroitin sulfate, undenatured collagen, and hyaluronic acid are variably effective as nutritional supplement ingredients; however, no clinical study has investigated the value of adding nucleotides to a formulation containing those active ingredients. The authors conducted an in vitro study on cultured chondrocytes using concentrations that simulate those in an actual nutritional supplement.*

***Materials and Methods:** The study assessed chondrocyte proliferation (viable cell count after trypan blue staining), chondrocyte vitality (MTT assay), collagen and glycosaminoglycan production (Sirius Red and Blyscan dye-binding assays, respectively), and TNF-α levels. The multi-ingredient formulation (glucosamine hydrochloride, bovine chondroitin sulfate, nucleotides, sodium hyaluronate, collagen, hyaluronic acid) was tested at concentrations of 0.63%, 1.25%, and 2.5%.*

***Results:** The highest concentrations of the formulation were variably associated with increased chondrocyte proliferation and mean collagen content in wells at 24 and 48 hours, whereas all concentrations were associated with increased glycosaminoglycan synthesis and no effects on TNF-α levels in the wells.*

***Discussion:** The solution containing nucleotides and other ingredients with acknowledged regenerative potential for chondrocytes, such as glucosamine, chondroitin sulfate, and undenatured collagen, that simulates a marketed nutritional supplement, promoted in vitro chondrocyte proliferation and de novo synthesis of collagen (at the two higher doses) and of glycosaminoglycans (at all dose levels) without pro-inflammatory effects.*

Keywords

Joint health, Chondrocyte proliferation, Chondrocyte vitality, Nucleotides.

Introduction

Protecting joint health in amateur practitioners of physical activities: the numbers depict a demanding task

A query to Claude Sonnet 5, an artificial intelligence (AI) research assistant, performed on 25 July 2026 — *"Can you give an estimate of amateur sports practitioners in Europe and North America?"* — yielded useful leads. Figure 1 illustrates the web sources the AI research assistant consulted to compile its preliminary suggestions, cross-checking them against those primary sources [1]. Although it used many official sources to generate its suggested leads, the large language model (LLM) research assistant noted the caveats that apply to AI-generated leads in academic contexts, especially

the need for direct verification, as well as the conventions for citing its methodological contribution (Figure 2) [1].

According to AI leads cited by the LLM, Eurostat estimates that about 44% of the EU population engaged in some physical activity at least once a week in 2019, though only about a third spent at least 150 minutes per week on aerobic activity [2]. A separate Eurobarometer survey cited by AI found that the share of Europeans who say they never exercise or play sport rose from 42% to 46%, meaning roughly 54% participate to some degree [3]. These estimates apply to the EU-27 only (about 380 million people age 15 or older). The AI research assistant cautiously extrapolated its findings to about 160-170 million regular participants in amateur sports. Wider Europe (EU + United Kingdom, Norway, Switzerland, Balkans, etc.), with about half a billion people, may count about 200-225 million amateur sport practitioners; and up to 250 million people, including Russia and Ukraine currently at war [1]. For the United States, the SFIA (Sports & Fitness Industry Association, a leading trade association) 2025 Topline Participation Report (released in February 2025 and reporting on 2024 activity) estimated that 247.1 million U.S. people participated in at least one sport or fitness activity in 2024, an 80% participation rate [4]. Factoring in Canada — 55% of Canadians aged 15 or older participating in organized sport [5] — and depending on how strictly "sport" is defined, a reasonable single-number global extrapolation may sum to roughly 400-450 million amateur sport practitioners across Europe and North America, out of a combined population of around 1.1-1.15 billion. This is no more than an AI

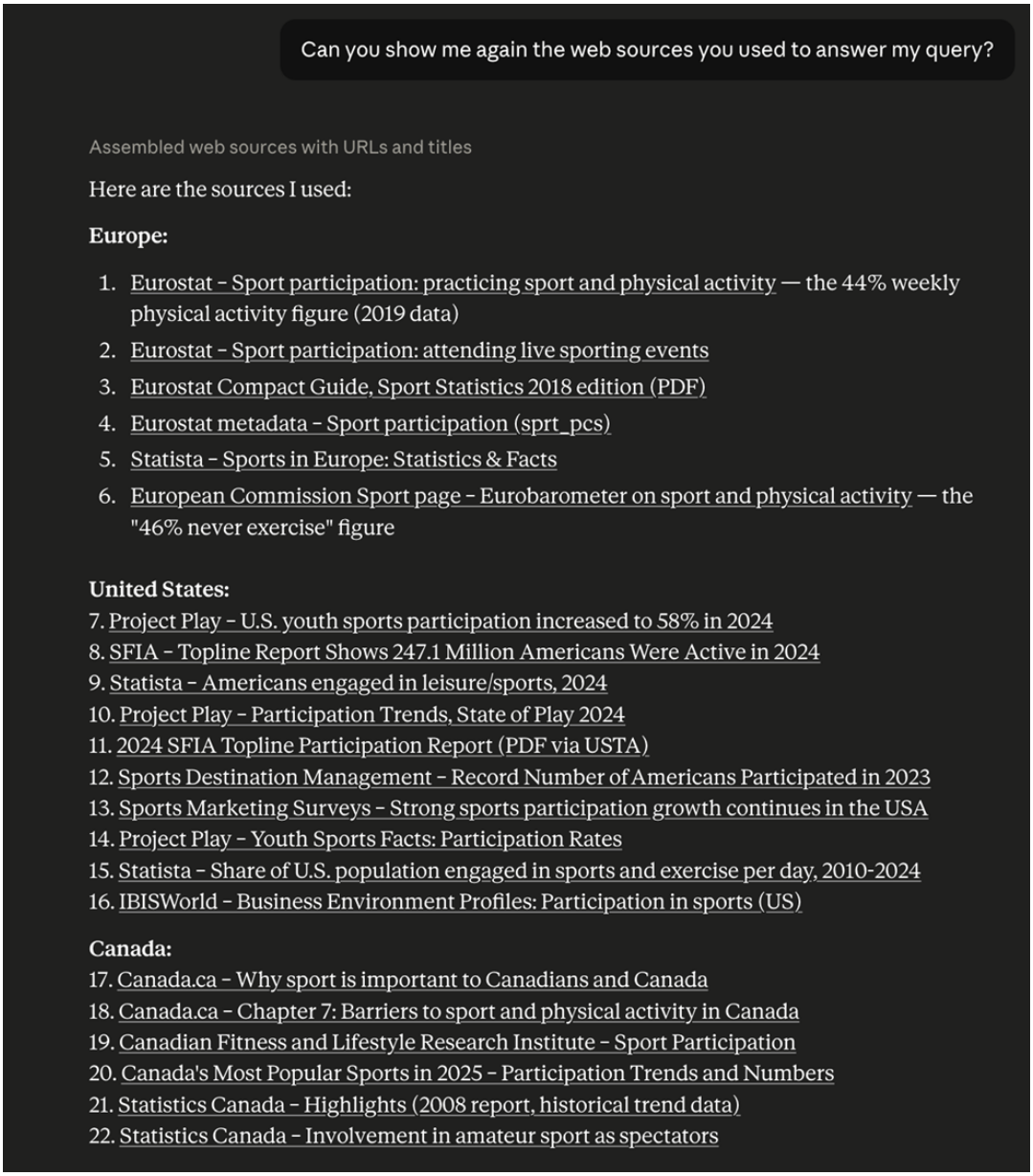

Figure 1: The web sources consulted by the AI model to answer the query about estimated amateur sports participation in Europe and North America. Actual screenshot of the leads suggested by AI [1].

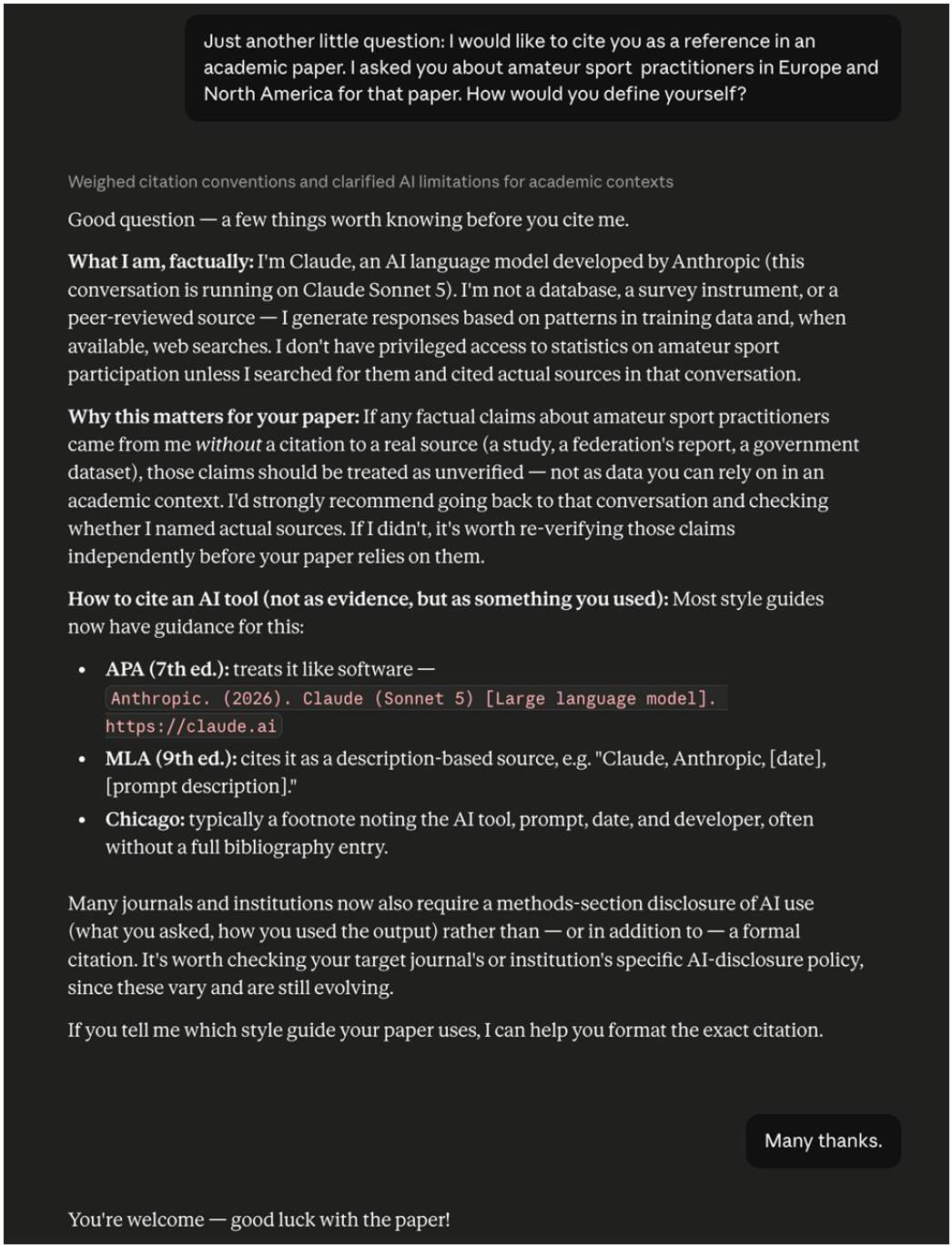

Figure 2: Cautions expressed by the language model itself regarding the use of AI-generated leads in academic contexts, including manuscripts submitted for publication, and guidance on how to cite the AI's methodological contribution. Actual screenshot of the AI's response to queries [1].

extrapolation based on different instruments, definitions, and years [1]. Still, it suggests that between a third and just under half of the European and North American populations may be involved in amateur sports.

These projections suggest that the global consumer market for joint health and joint protection is enormous — even larger worldwide, since the extrapolations exclude many other high- and middle-income regions in Asia, Latin America, and elsewhere. Nutritional supplements and their ingredients — for instance, glucosamine, chondroitin sulfate, collagen, hyaluronic acid, and, more recently, nucleotides — play a central role in this market.

How realistic is trying to protect joint health in sports practitioners?

The task does not appear easy. Take soccer, for instance — a professional and amateur sport with hundreds of millions of devotees worldwide. A randomized, double-blind, placebo-controlled study of soccer athletes with similar exercise burdens found that stand-alone glucosamine at 2 g daily for 12 weeks

significantly decreased the cartilage degradation marker urine C-terminal telopeptide-II (CTX-II) without affecting the cartilage synthesis marker carboxy-propeptide of type II procollagen (CPII), suggesting a chondroprotective effect [6]. However, a previous (2007) randomized, double-blind trial in healthy male athletes with acute knee injuries who took stand-alone glucosamine (1.5 g per day) or placebo for 28 days, with tracking of pain and functional recovery (passive knee flexibility in flexion and extension), found no significant difference between glucosamine and placebo [7].

A tentative conclusion is that oral chondroprotection with single-ingredient supplementation is likely unpredictable. More consistent benefits are likely when glucosamine sulfate and chondroitin sulfate, the two most common symptomatic, slow-acting drugs for osteoarthritis (SYSADOAs), are taken together [8]. Glucosamine exposure appears to be associated with inhibition of phospholipase A2, matrix metalloproteinases, and aggregases, while chondroitin appears to significantly decrease collagenolytic activity and enhance proteoglycan production, possibly in an additive manner [8].

The search for joint health and protection extends beyond sports-related injuries

Joint disorders, or simply joint fatigue, are widespread problems in the general population. The first culprit is osteoarthritis (OA), a degenerative inflammatory disorder of joint cartilage. The 2021 Global Burden of Disease study estimated that more than 600 million people worldwide had symptomatic, radiographically confirmed OA, primarily but not exclusively affecting arthrodial joints, representing roughly 7.7% of the global population. Age-standardized rates rose by 8.9% between 1990 and 2021, with knee OA alone accounting for 374.7 million cases worldwide in 2021 [9].

Even advanced Bayesian multiple-treatment network meta-analyses that account for random effects and uninformative priors—more precise than pairwise meta-analyses—have only partially confirmed claims that prescription-grade glucosamine sulfate—and, less consistently, other glucosamine salts—and chondroitin sulfate alleviate OA clinical symptoms (pain, joint space narrowing) at high doses or in combination. Moreover, uncertainty remains about the estimated effect size for pain change compared with placebo, despite the Bayesian approach's discriminatory power—integrating evidence from direct and indirect comparisons, preserving randomization, allowing simultaneous analysis of effect sizes reported at different follow-up times in a single model, and estimating the overall effect of preparations irrespective of follow-up observation while accounting for potential variation across time points [10]. Other advanced meta-analyses have failed to detect significant effects of minimal clinical relevance on pain or joint space narrowing compared with placebo [11].

Which other nutritional supplement ingredients may support joint health and protection?

Exposure to patented undenatured type II collagen from sternum cartilage, containing low levels of native collagen, appears to induce oral antigen tolerance and downregulate the immune response to joint collagen, which is linked to articular cartilage degeneration, synovitis, and bone remodeling [12]. The interaction of intact collagen antigen with intestinal lymphoid tissue is postulated to downregulate low-grade joint inflammation associated with OA or chronic sports-related trauma, helping control pain and joint discomfort during physical activity in still-healthy individuals and, speculatively, even enhancing cartilage repair or slowing further joint damage. Methodologically sound studies have confirmed the protective benefits of undenatured type II collagen in healthy and OA joints [12-14].

A randomized clinical trial comparing hyaluronic acid (HA) with placebo found that stand-alone HA doses below 150 mg did not affect cartilage damage, pain, stiffness, or physical function, as measured by WOMAC scores [15]. Thus, it is reasonable to include HA in oral, multi-ingredient nutritional supplements for chondroprotection and joint health; lower doses can act additively with other ingredients, such as medium-dose SYSADOAs. A Taiwanese double-blind, placebo-controlled, 8-week study evaluating an oral combination of glucosamine (750 mg), chondroitin sulfate (250 mg), and low-molecular-weight HA (50 mg) appeared to improve WOMAC pain and physical function subscores as early as two weeks after the first dose [16].

Nucleotides may also have a role in multi-ingredient chondroprotective oral supplements

Based on prior considerations, even accounting for confounding factors such as the natural course of joint problems, regression to the mean, and the placebo effect, there is a rationale for an oral nutritional co-formulation combining SYSADOAs, undenatured type II collagen, and low-dose hyaluronic acid to protect joints in subjects with known OA or at risk of joint damage, including athletes and amateur sports practitioners [17].

Nucleotides, widely used in nutritional supplements, may also serve as ingredients in joint health and chondroprotection supplements for the general population and among sports practitioners. Nucleotides are distinct from polynucleotides, which are used in medical devices for intra-articular administration and are derived from the DNA of salmon trout gonads. Conversely, nucleotides are produced by controlled hydrolysis of RNA from *Saccharomyces cerevisiae*.

Several studies in recent decades, up to 2024, have investigated the clinical efficacy of parenteral PN HPT, a proprietary, highly purified polynucleotide ingredient with characteristics similar to those of the nucleotides under investigation, either alone or in combination with HA, for gonarthrosis and other arthrosis-affected joints. These studies show that this ingredient provides persistent viscosupplementation, helps re-establish normal mesenchymal cell function, including that of chondrocytes, and is associated with more effective and more rapid pain reduction than HA alone [18-32]. Other preclinical investigations [33-36] illustrate additive *in vitro* and *in vivo* effects on how fibroblasts, synoviocytes, and chondrocytes respond to nucleotides and HA. Two reviews summarize these findings [37,38]. After oral intake, small, short-stranded DNA oligopolynucleotides resist degradation by gastric

juice and are modestly absorbed, with low bioavailability, whereas nucleosides are absorbed more readily and can replenish and fortify the body's pools, including in the joints; polynucleotide uptake via the lysosomal/intracellular route mediated by the SIDT2 protein could be another possibility [39,40]. Thus, there is nothing to prevent the use of nucleotides as an ingredient in a nutritional supplement. Currently, no well-designed clinical study supports the rationale for a complex chondrocyte-protective combination that includes nucleotides and more consolidated ingredients such as glucosamine, chondroitin sulfate, undenatured collagen, and hyaluronate. A preclinical study might be a useful first step. The *in vitro* study described herein investigated whether a combination of nucleotides, bovine chondroitin sulfate, glucosamine hydrochloride, sodium hyaluronate, and undenatured collagen could stimulate the trophism of cultured human chondrocytes, either synchronized in the G0 (quiescent) phase of the cell cycle or at active confluence. The contributions of the different ingredients are sound: nucleotides and hyaluronic acid may help maintain the physiological integrity of connective tissues, while chondroitin sulfate, collagen, and glucosamine are essential components of the joint cartilage's extracellular matrix and synovial fluid. The authors evaluated de novo synthesis of collagen and glycosaminoglycans (GAGs) and the activity of the acute-phase proinflammatory TNF-α cytokine at 24 and 48 hours.

Materials and Methods

Chondrocyte proliferation study

Human chondrocytes from a commercial source (chondrocyte human cell culture, Cell Applications, San Diego, CA, USA; cat 402-05a, lot 3507), with confirmed production of aggrecan and type II collagen by fluorescence-activated cell sorting and Western blot analysis, were seeded in a triple set of 96-well plates (VWR 734-2803, Batch: 220618078B, VWR, Milan, Italy; seeding concentration: 5 x 10^5 cells per mL) containing incomplete DMEM (Dulbecco's Modified Eagle Medium without fetal bovine serum, Biowest, Nuaillé, France). To induce cellular quiescence, chondrocytes were cultured under standard conditions in a thermostatically controlled CO_2 Galaxy S Plus incubator (RS Biotech Laboratory Equipment Ltd, Irvine, Scotland); in a thermostatic bath (Grants Instruments Ltd, Royston, UK); and within a laminar airflow cabin with HEPA filters (ASALAIR 1200, Asal Srl, Cernusco sul Naviglio, Italy), with a constant flow of 5% CO_2 (v/v in air) and constant conditions of 37 ± 1°C and 90 ± 10% relative humidity (HR), controlled by a data logger incubator. After 24 hours, the quiescent chondrocytes, now reseeded in complete DMEM (fetal bovine serum: Sigma-Aldrich, Milan, Italy) to restart the cells from the G0 phase of the cell cycle, were exposed to scaled concentrations (2.5%, 1.25%, and 0.63%) of a solution of ingredients proportionally matching the composition of a marketed joint health nutritional supplement (Condrotide MOVEO, Mastelli Srl, Sanremo, Italy; composition described in Supplementary Table A). Exposure followed a preliminary cytotoxicity test to select the three doses to be tested (incubation of cell suspensions with 5% trypan blue, which stains nonviable cells blue; Sigma-Aldrich, Milan, Italy) for 5 minutes at room temperature. Chondrocyte proliferation was assessed by counting 10 µl of the stained cell suspension in a specific chamber under an optical microscope (Nexcope, Nanjing, China) at 20X and 40X magnification. Negative controls were cultured in complete DMEM only. After the scaled exposures, the medium was removed from the wells by gentle suction using multichannel mechanical pipettes (VWR, Milan, Italy). An MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was then performed to evaluate cell viability and the proliferation index relative to the untreated control, with staining defined as 100% viability.

MTT assay

The MTT test (Sigma-Aldrich, Milan, Italy) is a standard colorimetric method for assessing cell viability by measuring the reduction of yellow tetrazolium salts to blue-purple formazan crystals by mitochondrial succinate dehydrogenase in viable cells. At the end of the exposure period to the scaled-dose test solutions, the plate wells were rinsed with standard PBS (phosphate-buffered saline solution), stained with a 1-mg/mL MTT solution, and incubated in a thermostat for three hours under standard conditions (37 ± 1°C, 5% CO_2, 90 ± 10% RH). Isopropanol was added to each well, and the plates were agitated for 2 hours at room temperature. Finally, 100 µL of dimethyl sulfoxide was added to each well, and the plate was shaken for 10 minutes at room temperature to dissolve the formazan crystals.

Absorbance readings were measured spectrophotometrically at 570 nm using a microplate reader for ELISA plates (Biotek Synergy LX, Biotek, Milan, Italy); isopropanol served as the blank. Each experiment was repeated at least three times, and the viability rate was defined as the ratio of the average optical density of treated cultures to that of negative controls, with MTT reduction considered 100% cell viability.

Collagen and GAG assays

In parallel with the proliferation and viability assays, another set of three confluent human chondrocyte cultures was seeded in a 96-well plate and treated with the test solution at scaled concentrations, as previously described. Specifically, cells were incubated in a thermostatically controlled incubator under standard conditions for 24 hours or until reaching confluence. At the end of the incubation period, the cells were exposed to the test product for 24 and 48 hours at concentrations of 2.5%, 1.25%, and 0.63%. All procedures, including those for negative test controls, were performed as previously described.

Collagen assay (quantitative Sirius Red dye-binding method)

Sirius Red (Direct Red 80) — an anionic dye with sulfonic acid side groups that interact with the basic amino acids of native, non-denatured collagen with an intact triple-helical structure — was used as the chromogen in the dye-binding assay. Its specificity for collagen results from the alignment of the elongated dye molecules parallel to the rigid structure of native collagen.

Collagen quantification was performed according to the

manufacturer's instructions. Briefly, 1.0 mL of Sircol Dye Reagent (Biocolor Ltd, Belfast, UK) was added to each sample and each collagen standard. The triple set of samples was incubated for 30 minutes to allow the collagen-dye complex to form and precipitate from the unbound dye. After incubation, the samples and standards were transferred to microcentrifuge tubes and centrifuged at 13,000 × g for 10 minutes. The supernatant was discarded, and 750 µL of ice-cold Acid-Salt Wash Reagent (same manufacturer) was added to each collagen-dye pellet to remove unbound dye from the pellet surface. The samples were then centrifuged again at 13,000 × g for 10 minutes, after which the supernatant was discarded.

The collagen-bound dye was released by adding 1,000 µL of Alkali Reagent (same manufacturer) to each sample. After the bound dye was fully solubilized, the absorbance of the samples and standards was measured at 556 nm using a microplate reader. Absorbance data were interpolated from a standard calibration curve spanning increasing collagen concentrations.

GAG assay (quantitative Blyscan dye-binding method)

The 1,9-dimethylmethylene blue dye labels the sulfated polysaccharide proteoglycan fraction and protein-free sulfated glycosaminoglycans specifically and quantitatively. Quantification was performed according to the manufacturer's instructions. Briefly, 1.0 mL of Blyscan Dye Reagent (Biocolor Ltd, Belfast, UK) was added to each sample. The samples were incubated for 30 minutes to allow precipitation of the sulfated glycosaminoglycan-dye complex from the soluble unbound dye, then centrifuged at 13,000 × g for 10 minutes, followed by a second centrifugation for 5 minutes. The resulting supernatants were discarded. Subsequently, 0.5 mL of Dissociation Reagent (same manufacturer) was added to each tube to solubilize the bound dye. Once the dye was completely dissolved, absorbance was measured at 650 nm using a microplate reader, and the data were interpolated against a standard calibration curve spanning increasing GAG concentrations.

TNF-α assay (ELISA)

A commercial, peroxidase- and plate-based Enzyme-Linked Immunosorbent Assay (TNFALPHA KIT, Cloud-Clone Corp., Houston, USA) was used to quantify levels of the acute-phase proinflammatory cytokine TNF-α, a marker of inflammation, in culture media at the end of the experimental follow-up. The assay was also performed in triplicate. Wells were assigned to diluted standards, blank, and test samples, with seven wells prepared for the standard curve and one well for the blank. 100 µL of each standard dilution, blank, and test sample was added to the corresponding wells. Standard ELISA procedures were followed, including detection reagent working solutions, substrate solution, stop solution, multiple washing cycles, and final yellow color development. The proinflammatory antigen was quantified spectrophotometrically at 450 nm using a microplate reader by interpolating absorbance data against a standard calibration curve spanning increasing TNF-α concentrations.

Statistics

Sequential results at 24 and 48 hours were analyzed using one-way ANOVA to assess differences in the outcome distribution relative to pre-treatment (baseline). If overall differences were detected, a post hoc Tukey's test compared corresponding marker values (samples vs. negative controls) to detect differences across repeated assessment times. A p value less than 0.05 was considered statistically significant.

Results

Figure 3 presents the comparative results of the chondrocyte proliferation and viability study at 24 and 48 hours. Exposure to the highest concentrations (1.25% and 2.5%) of the solution containing nucleotides and other ingredients with regenerative potential for chondrocytes did not affect chondrocyte viability and significantly increased chondrocyte proliferation after 24 hours in a dose-dependent manner (mean cell counts across the active wells), compared with negative controls — +29.2% and +39.8%, respectively. The proliferative effect at 0,63% exposure (+8.1%) was non-significant.

No adverse effects on cell viability were observed at any exposure level after 48 hours, but the proliferative effects on chondrocytes were no longer significant. The reference mean proliferation of negative controls was conventionally set at 100. The corresponding means ± standard deviations were 108.1 ± 7.9%, 129.2 ± 9.2%, and 139.8 ± 17.8% for the 0.63%, 1.25%, and 2.5% concentrations, respectively, at 24 hours, and 101.3 ± 4.5%, 104.2 ± 11.5%, and 107.1 ± 3.6% for the 0.63%, 1.25%, and 2.5% concentrations, respectively, at 48 hours.

Figure 4 shows the mean collagen content (micrograms per well), averaged across active wells with scaled test formulations at 24 and 48 hours, compared with negative controls. The lowest concentration (0.63%) did not affect collagen content, with non-significant increases of +3.5% at 24 hours and +2.9% at 48 hours. By contrast, exposure to the highest concentrations (1.25% and 2.5%) significantly increased mean collagen content at 24 hours in active wells in a dose-dependent manner — +198.6% and +426.8%, respectively. A statistically significant effect on collagen production persisted after 48 hours only at the 2.5% dose level (+310.7%).

Figure 5 shows the mean GAG content (micrograms per well), averaged across active wells at 24 and 48 hours, compared with negative controls. Results are presented as absolute concentrations (upper table) and as percent changes (lower graph). Exposure to all tested concentrations was associated with increased GAG synthesis at both 24 and 48 hours relative to negative controls.

Figure 6 presents the mean TNF-α content at 24 and 48 hours (nanograms per well), averaged across wells containing the tested solutions, compared with negative controls. Results are shown as absolute concentrations (upper table) and as percent changes (lower graph). No exposure to any tested concentrations was associated with changes in pro-inflammatory cytokine levels in the plate wells.

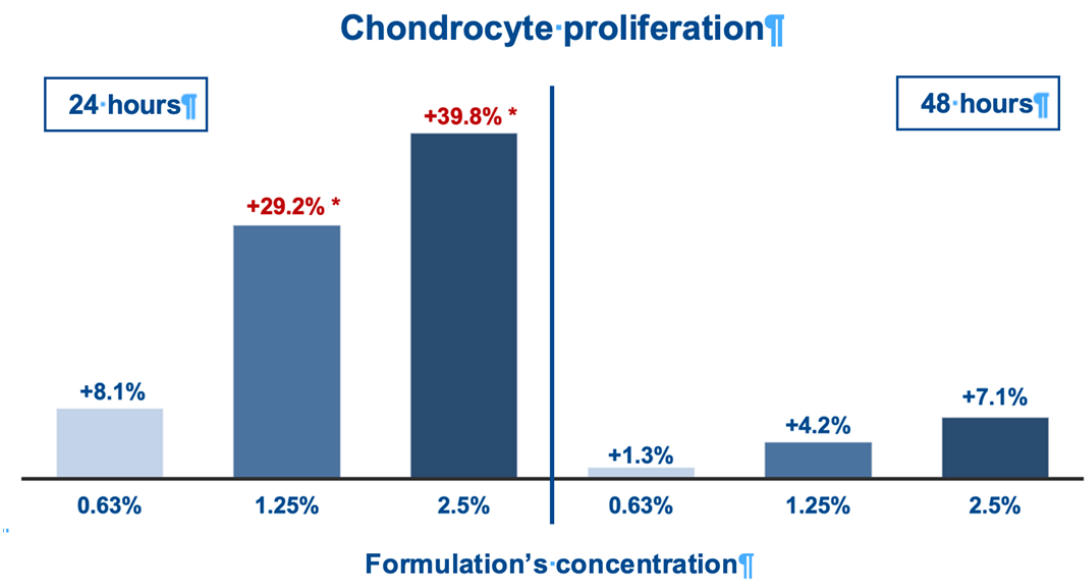

Figure 3: Effects on *in vitro* chondrocyte proliferation after 24 and 48 hours of exposure to increasing concentrations of the nucleotide-containing formulation compared with negative controls. Results are expressed as the percent change in mean chondrocyte density across wells with scaled-dose formulations relative to negative controls. Asterisks and vermilion numbers indicate statistical significance ($p < 0.05$) vs. negative controls.

Mean collagen content per well

24 hours	*Negative controls*	2.0 ± 0.78
	Concentration 0.63%	2.7 ± 0.78
	Concentration 1.25%	**6.0 ± 0.48 ***
	Concentration 2.5%	**10.6 ± 0.45 ***

48 hours	*Negative controls*	4.0 ± 0.27
	Concentration 0.63%	4.4 ± 0.66
	Concentration 1.25%	4.8 ± 0.97
	Concentration 2.5%	**16.3 ± 1.02 ***

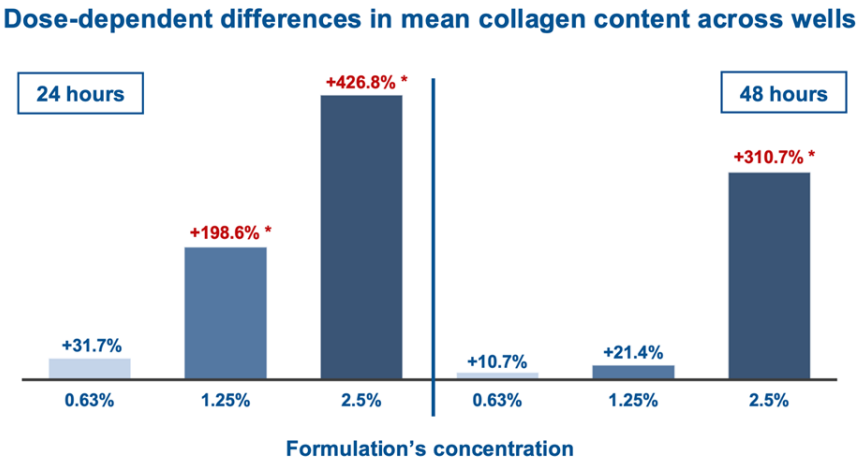

Figure 4: Effects on mean collagen content at 24 and 48 hours, averaged across wells with increasing concentrations of the nucleotide-containing solution, compared with negative controls. Results are expressed in the upper table as the mean collagen content (micrograms per well) and standard deviations across active wells relative to the mean collagen content in negative-control wells; in the lower graph as percent differences vs. negative controls. Asterisks and vermilion numbers indicate statistical significance ($p < 0.05$) vs. negative controls.

Mean glycosaminoglycan content per well

24 hours	*Negative controls*	0,7 ± 0.04
	Concentration 0.63%	**1.0 ± 0.05 ***
	Concentration 1.25%	**1.1 ± 0.08 ***
	Concentration 2.5%	**1.5 ± 0.17 ***

48 hours	*Negative controls*	1.1 ± 0.04
	Concentration 0.63%	**2.0 ± 0.05 ***
	Concentration 1.25%	**2.3 ± 0.11 ***
	Concentration 2.5%	**2.0 ± 0.05 ***

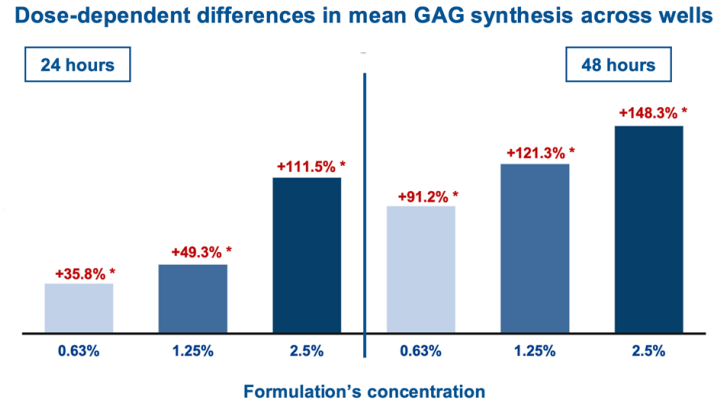

Figure 5: Effects on mean glycosaminoglycan (GAG) synthesis and content in plate wells at 24 and 48 hours, averaged across wells with increasing concentrations of the nucleotide-containing solution, compared with negative controls. Results are expressed in the upper table as absolute-value means ± standard deviation of GAG content (micrograms per well) relative to the mean GAG content in negative-control wells; in the lower graph as percent differences relative to negative controls. Asterisks and vermilion numbers indicate statistical significance ($p < 0.05$) vs. negative controls.

Discussion and Conclusions

Extensive preclinical experience with the previously cited proprietary, highly purified ingredient, which has characteristics analogous to those of the nucleotides under investigation, along with a similarly broad clinical research program using parenteral (subdermal and subcutaneous) formulations, demonstrates nucleotide regenerative effects on mesenchymal cells, including chondrocytes and fibroblasts [33-36,38,41-43]. Although these studies used a similar ingredient, they provide a solid foundation for biological plausibility. In particular, a previous preclinical study using cartilage biopsy specimens from patients with osteoarthritis reported stimulation of chondrocyte growth and restoration of cartilage matrix-related functions, including the deposition of collagen type II and aggrecan [33].

This study, which involved in vitro exposure of cultured human chondrocytes to a complex solution of nucleotides and other ingredients with acknowledged regenerative potential for chondrocytes, confirmed earlier findings. The association of nucleotides with glucosamine, chondroitin sulfate, and undenatured collagen mirrors the formulation of a marketed nutritional supplement for joint health, used either alone or associated with polynucleotide-based medical devices for intra-articular administration (see Supplementary Table 1). Nucleotides have a long history of use in nutritional supplements, including infant formulas, with full European and American regulatory approval, which helps them better mirror human milk composition [44-46]. Nucleotide-based nutritional supplements are also widely used among professional and amateur sports practitioners and in the general population, with demonstrated protection of joints and improved muscle cell energy metabolism and contractility [47,48]. Consistent with these findings, exposure to the tested formulation was associated with enhanced chondrocyte proliferation, with a significant effect in the MTT assay after 24 hours at the two highest concentrations (2.5% and 1.25%). The proliferative response appears mainly associated with early exposure, as no enhanced chondrocyte proliferation was apparent at 48 hours.

In vitro exposure to the multi-ingredient formulation also affected extracellular matrix-related parameters, with significant, concentration-dependent increases in collagen synthesis at 24 hours at both 2.5% and 1.25%; the effect persisted at 48 hours at 2.5%. In parallel, GAG synthesis increased significantly and in a concentration-dependent manner at both time points across all tested concentrations. These findings suggest that the formulation may support chondrocyte activity and promote the synthesis of key components of the cartilage extracellular matrix.

The study showed a neutral inflammatory profile for the tested formulation. The slight reduction in TNF-α levels, up to about 10%, was not statistically significant.

Mean TNF-α content per well

24 hours	*Negative controls*	6.0 ± 0.05
	Concentration 0.63%	5.8 ± 0.19
	Concentration 1.25%	5.9 ± 0.33
	Concentration 2.5%	5.7 ± 0.19

48 hours	*Negative controls*	6.7 ± 1.0
	Concentration 0.63%	6.6 ± 1.19
	Concentration 1.25%	6.3 ± 1.19
	Concentration 2.5%	6.6 ± 0.87

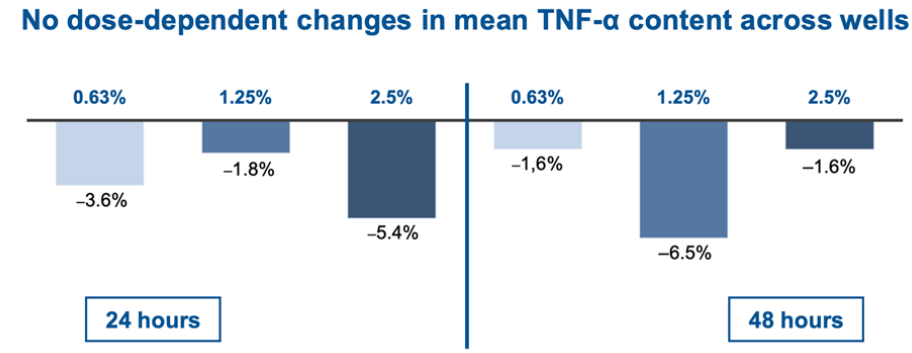

Figure 6: Effects on mean TNF-α levels in plate wells at 24 and 48 hours, averaged across wells with increasing concentrations of the nucleotide-containing solution, compared with negative controls. Results are expressed in the upper table as absolute-value means ± standard deviation of TNF-α levels (nanograms per well) relative to the mean TNF-α levels in negative-control wells; in the lower graph as percent differences relative to negative controls. No statistical significance vs. negative controls.

Because of its design, the study cannot attribute the observed favorable effects to any specific component of the multi-ingredient formulation. However, its findings suggest that a nucleotide-containing nutritional supplement mirroring the tested formulation may support chondrocyte function and vitality and stimulate the synthesis of cartilage-associated extracellular matrix components, including collagen and GAGs, without pro-inflammatory effects and with an early effect within the first 24 hours. Still, *in vitro* results cannot be directly extrapolated to clinical settings. Only well-designed mechanistic and clinical studies will determine whether these *in vitro* results translate into meaningful benefits for joint health.

Ethical Statement

The steering committee of the private laboratory where the in vitro study was conducted under the authors' supervision approved the study, waiving the requirement for further Institutional Review Board approval.

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Supplementary Table 1: Condrotide MOVEO (Mastelli Srl, Sanremo, Italy), ingredients. q.s. = quantum satis (sufficient quantity).

Condrotide MOVEO, ingredients

Nucleotides	100 mg
Glucosamine	415 mg
Chondroitin sulfate	360 mg
Hyaluronic acid	30 mg
Type II Collagen of which native	10 mg 4 mg
Hydroxypropyl cellulose, cellulose, dicalcium phosphate, mono- and diglycerides of fatty acids, magnesium stearate, silicon dioxide	q.s.
TOTAL	**1450 mg**

Gluten- and lactose-free

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