2cool®

NATIVE TYPE II COLLAGEN

FINDING A POINT OF DIFFERENCE IN THE **EVOLVING COLLAGEN SUPPLEMENTS** MARKET

Bioiberica

ABSTRACT

Recent insights reveal that the collagen market is expanding, creating exciting opportunities for dietary supplement manufacturers to innovate in the joint health sector. However, staying competitive in this evolving arena is challenging, especially when looking to develop novel solutions with widely researched ingredients that are also easy to formulate. This whitepaper discusses evidence that native (undenatured) type II collagen is effective in supporting joint health at lower doses, therefore meeting increasing consumer demand for convenient products that support their health.

JOINT HEALTH & MOBILITY: AN EVOLVING MARKET

Joint health is now an important public health concern across the globe, largely due to the ageing population. Age can significantly impact our muscles, bones and joints; 45% of individuals aged 65+ say they experience joint pain, which affects their overall mobility and independence.[1] Furthermore, staying fit and active as we age are increasingly important health focuses – especially for senior consumers who are taking a more proactive approach to supporting their joint health. However, consumers of all ages can be affected by joint discomfort. Several reports, for instance, demonstrate that sporty people, the 40+ population and women experiencing menopause commonly experience joint discomfort or mobility issues.[2,3,4] These trends have given significant momentum to the joint health sector and are a major driving force in the emergence of innovative joint health solutions. Between 2019 – 2024 alone, it is forecast that the global bone and joint ingredients market will grow at a CAGR of 6.3% to meet this demand.[5]

COLLAGEN: DRIVING GROWTH IN THE JOINT HEALTH CATEGORY

As well as the ageing population and trend towards staying active and healthier for longer, ingredients are also driving growth in the joint health segment. Glucosamine and chondroitin have long been used as active ingredients for joint health. However, other innovative ingredients, such as collagen, are now gaining rapid market share as a result of rising consumer awareness, driving significant growth in the category. According to recent market data, sales in the joint health market increased by 4.3 % in 2018 in the US alone, largely driven by a boost in collagen sales, which increased by 30%.[6] As a result, the joint health category is seeing its best overall growth since 2008.

TYPE II COLLAGEN: THE MAIN STRUCTURAL PROTEIN IN CARTILAGE

Collagen is the main component of connective tissues that make up tendons, ligaments, skin and cartilage. Although it has many important functions in the body, collagen is best known for its structural role – providing a structural framework for tissues throughout the body.[7] Of the 28 different types of collagen that have been identified, type II collagen is the main structural protein in cartilage. Both native (undenatured) type II collagen and hydrolysed (denatured) collagen are available for commercial use in joint health products. However, there are significant differences between the two forms.

DID YOU KNOW?

Native type II collagen and undenatured type II collagen are the same molecule, but known by different terms throughout the joint health category.

Native type II collagen – also known as undenatured or non-hydrolysed type II collagen throughout the nutrition industry – is collagen in its biologically active form.

Hydrolysed collagen – or denatured collagen – is collagen that has been broken down into smaller peptide molecules.

NATIVE TYPE II COLLAGEN VS. HYDROLYSED COLLAGEN: WHAT'S THE DIFFERENCE?

In its natural form, collagen has a folded triple helix structure consisting of long polypeptide chains (see figure 1). Hydrolysed collagen is manufactured via a specific hydrolysis process, where enzymes "cut" the triple helix molecule into smaller pieces, i.e. short-chain peptides. This is why hydrolysed collagen is also known as collagen peptides, or denatured type II collagen.

Native type II collagen on the other hand, is not hydrolysed and maintains its characteristic three-dimensional structure.

Figure 1: The triple helix structure of native (undenatured) type II collagen

DIFFERENT MECHANISMS OF ACTION

The mechanism by which each collagen acts differs. Native (undenatured) type II collagen works via an immune-mediated process, known as oral tolerance. Through this mode of action native type II collagen is recognised by the immune system as a known substance and deactivates the body's immune response against its own collagen. Alternatively, hydrolysed collagen peptides are highly bioavailable, resulting in a source of the specific amino acids for *de novo* synthesis of collagen. As such, hydrolysed collagen peptides act as building blocks to maintain and rebuild cartilage.

EFFECTIVENESS AT LOWER DOSES

The daily dose and intake required for both collagens to be effective in the body varies greatly. The native (undenatured) type II collagen form is recommended at doses as low as 40 mg/day. Meanwhile, the recommendation for hydrolysed collagen is 10 g/day (see figure 2). The low dosage required for native type II collagen therefore mirrors consumer demand for easy-to-consume, convenient products, offering an innovative alternative to supplement manufacturers.

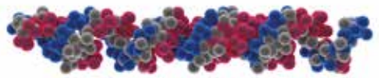
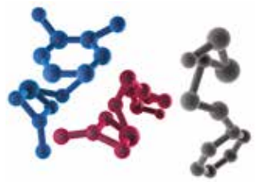
	NATIVE TYPE II COLLAGEN	HYDROLYSED COLLAGEN
MOLECULE	Native (undenatured) form - triple helix	Denatured - cut into small peptides
		
TYPES OF COLLAGEN	Type II (specific)	Non (specific)
ABSORPTION	No	Yes
MECHANISM OF ACTION	Immune mediated	Anabolic
MAIN EFFECT	Decrease of collagen destruction	Increase of collagen production
DOSE	40 mg	10 g

Figure 2: Native type II collagen vs. hydrolysed collagen

THE ROLE OF THE IMMUNE SYSTEM IN JOINT HEALTH

Joint disorders involving inflammation and cartilage erosion, such as arthritic diseases, are characterised by an autoimmune component in which the immune system acts against the body's own type II collagen.[8] Classically, osteoarthritis (OA) has been characterised as a degenerative, wear-and-tear disease. However, recent scientific research has identified it as an immunopathological disease – in other words, a disease in which the immune system plays a key role.

That is because in OA, products from collagen breakdown can be recognised by immune cells as potentially harmful. As a consequence, an immune response against collagen is activated, leading to inflammation and cartilage degradation, further damaging the joints.

CARTILAGE DEGRADATION & IMMUNE RESPONSE

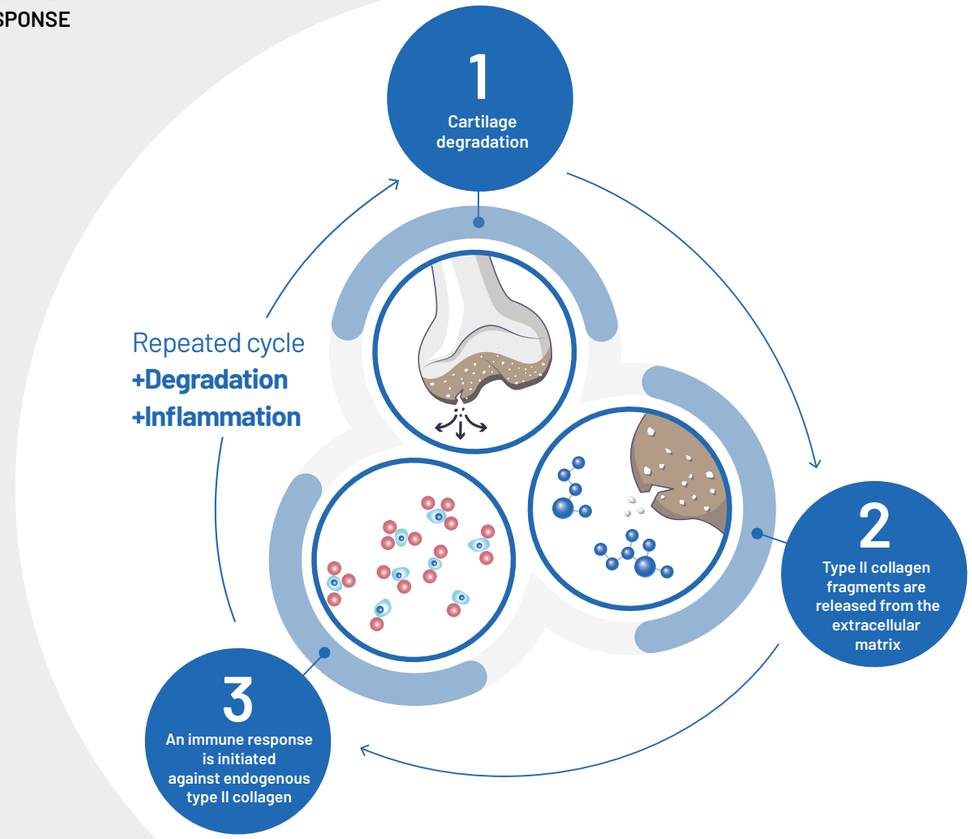


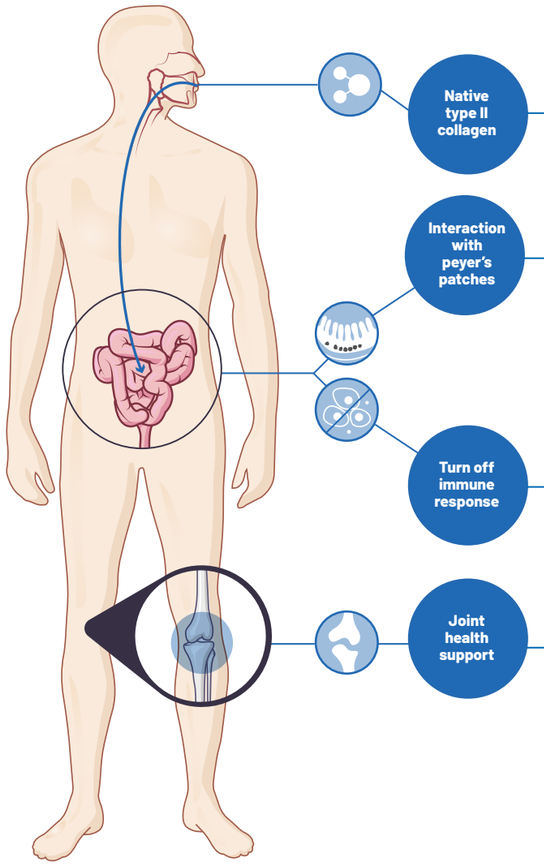
Figure 3: Autoimmune response to collagen breakdown

ORAL TOLERANCE:
AN IMMUNE-MEDIATED RESPONSE

Studies show that supplementing native (undenatured) type II collagen can help modulate the immune response against endogenous type II collagen, thus supporting joint health.[9]

Thanks to this specific mechanism of action, it takes just a small amount of native type II collagen to support joint health. This is why the standard dose of ingredients containing native collagen is just 40 mg, once daily, whereas dosages for hydrolysed collagen can be up to 10 g/day.

The positive immune modulation promoted by native collagen intake – its ability to prevent the immune response against type II collagen produced by the body – has been receiving increasing interest across the scientific community.



When taken orally (see figure 4):

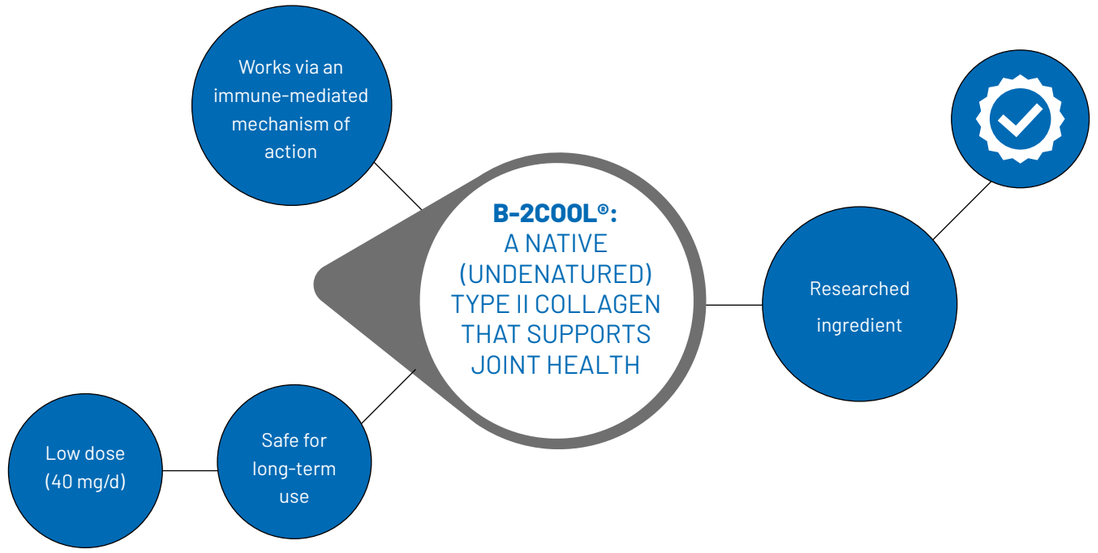
1. Native (undenatured) type II collagen passes through the stomach and reaches the intestine
2. It interacts with the Peyer's patches in the intestine, which are lymphoid nodules responsible for immune surveillance of the intestinal tract
3. It turns off the immune response against endogenous type II collagen
4. It reduces collagen degradation in the joint, supporting joint health

Figure 4: Native (undenatured) type II collagen reaches the Peyer's patches in the intestine where it turns off the immune response to endogenous type II collagen; reducing collagen degradation in the joint and supporting joint health

ORAL TOLERANCE IS THE MODE OF ACTION BY WHICH NATIVE (UNDENATURED) TYPE II COLLAGEN WORKS IN THE BODY.

INNOVATING WITH B-2COOL® NATIVE TYPE II COLLAGEN

To meet growing demand for more effective, low-dose solutions in the joint health market, Bioiberica has developed b-2Cool® - a widely researched, natural-origin ingredient that supplies native type II collagen to support joint health. Extracted from chicken sternum, the manufacture of b-2Cool® is strictly controlled to maintain its characteristic triple helix structure and the biologically active epitopes of the native protein. A low dose of only 40 mg/day of b-2Cool® is required to be effective, meeting consumer demand for convenient, low-dose products and reducing pill fatigue.



INSPIRING THE NEXT GENERATION OF JOINT HEALTH PRODUCTS

We're not just suppliers, we're industry partners. We provide the scientific, regulatory, industrial and market expertise to develop innovative, market-leading solutions that will help make a difference.

The efficacy of b-2Cool® supplementation has been assessed in two clinical studies (one single blind trial and one retrospective trial) in patients with OA, and in two preclinical studies using animal models of OA. Results from these studies showed that the ingredient effectively supports joint health.

THE SCIENCE BEHIND B-2COOL®

1. IN VIVO STUDY: EFFECT OF NATIVE (UNDENATURED) TYPE II COLLAGEN IN A RAT MODEL OF OSTEOARTHRITIS INDUCED BY MIA[10]

Mannelli LDC, et al. Low dose chicken native type II collagen is active in a rat model of osteoarthritis. Osteoporosis Int., 2015, vol. 26, pg. 184.

OBJECTIVE

To evaluate the role of low doses of chicken native type II collagen in the rat model of osteoarthritis, induced by sodium monoiodoacetate (MIA).

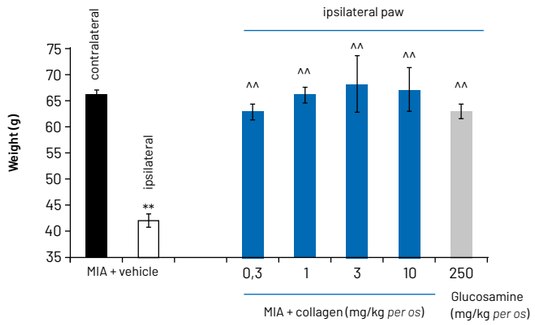
METHODS

0.3–10 mg/kg chicken native type II collagen was daily administered orally for 14 days starting from the day of MIA intra-articular injection. Glucosamine (250 mg/kg p.o.) was used as a reference compound. Pain behaviour measurements (paw pressure test; Plantar Test; Von Frey test; Incapacitance test; Animex test) were performed on days seven and fourteen. On day fourteen, plasma samples were collected to evaluate biochemical parameters.

RESULTS

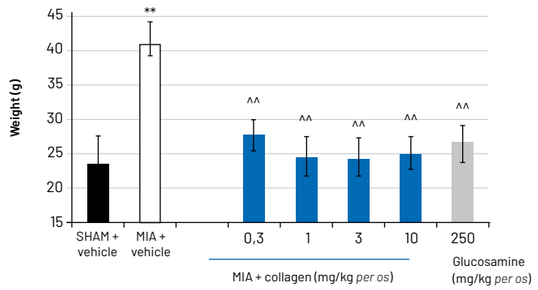
Native (undenatured) type II collagen (1–10 mg/kg) significantly reduced mechanical hyperalgesia (figure 5 paw pressure test) on days seven and fourteen. The lower dosage was effective on day fourteen. Efficacy was comparable to those induced by 250 mg/kg glucosamine. On day fourteen, collagen counteracted thermal hyperalgesia, as measured by the Plantar Test. Moreover, collagen significantly decreased the response to mechanical sensitivity (Von Frey test) both on days seven and fourteen. As evaluated by the Incapacitance test, collagen (1–10 mg/kg) was able to prevent MIA-induced spontaneous pain. Repeated treatment with collagen improved the spontaneous mobility of the animals, as evaluated by the Animex test. Also, native type II collagen was able to prevent the MIA-dependent plasmatic increase of IL-1ß (figure 6) and TNFα. Finally, repeated collagen administrations reduced the degradation of endogenous collagen since the plasmatic levels of the degraded fragment C2C were significantly decreased. The stimulus to a de novo synthesis of collagen (propeptide CPII) was maintained.

Figure 5: Paw pressure test



**P<0.01 vs sham + vehicle treated animals
^^P<0.01 vs MIA + vehicle treated animals

Figure 6: IL-1ßplasmatic levels on day fourteen



**P<0.01 vs sham + vehicle treated animals
^^P<0.01 vs MIA + vehicle treated animals

CONCLUSION: These results describe the effects of low dosages of chicken native type II collagen by a mechanism that involves a protective effect on cartilage.

2. IN VIVO STUDY: EFFECT OF NATIVE (UNDENATURED) TYPE II COLLAGEN ON GAG COMPOSITION IN A RABBIT MODEL OF OSTEOARTHRITIS[11]

Sifre V, et al. Macroscopic and histologic improvements in joint cartilage, subchondral bone and synovial membrane with glycosaminoglycans and native type II collagen in a rabbit model of osteoarthritis. Osteoarthritis Cartilage, 2020, vol. 28, pg. S206. Sifre V. et al. Glycosaminoglycans combined with native type II collagen improve magnetic resonance imaging biomarkers in a rabbit osteoarthritis model. Veterinary Surgery, 2020, vol 49, pg. O238–O239.

OBJECTIVE

To evaluate the effects of native type II collagen (NC) in combination with chondroitin sulphate (CS), glucosamine hydrochloride (GH) and a rooster comb extract rich in hyaluronic acid (HA) – in a rabbit model of osteoarthritis induced by anterior cruciate ligament section.

METHODS

Following osteoarthritis-inducing surgery, rabbits were divided into three groups (n=18). Each group received a daily oral administration, starting on the surgery day, of the following combination: Group 0 (control group) – no treatment. Group 1 – CS (CS b-Bioactive®) + GH + HA (Mobilee®). Group 2 – CS + GH + HA + NC (b-2Cool®). For cartilage, bone and synovial membrane evaluation, samples of lateral femoral condyle and synovial membrane were obtained after 28, 56 and 84 days.

Tibial plateau and femoral condyle images from Group 0 and Group 2 were obtained with a 3T MRI scanner. The non-operated knee from Group 0 was used as the healthy control.

RESULTS

Macroscopic evaluation showed significantly improved cartilage appearance in group two when compared to the other groups in the study, and was closer to that of healthy cartilage (Figure 7). Microscopically, the assessment of articular cartilage revealed significantly better cartilage structure, chondrocyte density, subchondral bone and synovial membrane for the treated groups, compared to the control group, indicating a lower degree of degenerative changes in the treatment groups.

Histologic evaluation of the synovial membrane showed significantly lower values in Group 2 compared to the other groups; and significantly lower values in Group 1 when compared to the untreated group.

MRI evaluation showed that, in Group 0, all biomarkers in the injured knee were significantly worsened compared to the healthy one. However, Group 2 showed better results compared to the control group and values closer to the healthy ones.

Overall, Group 2's joint structures showed values closer to those of a healthy joint, followed by Group 1. Whereas, the joints in the untreated group featured more advanced degenerative process of osteoarthritis.

The main findings are summarised in figure 7.

Figure 7: Summary of the main results obtained in the different study groups after 84 days.

Groups	Treatments	Improved cartilage appearence	Improved cartilage structure, chondrocyte density, subchondral bone and synovial membrane	Improved synovial membrane	Similarity to a healthy joint
0	None	–	–	–	–
1	**CS** - CS b-Bioactive® (chondroitin sulphate) + **GH** - (glucosamine) + **HA** – Mobilee® (rooster comb extract rich in hyaluronic acid)	+	+	+	+
2	**CS** + **GH** + **HA** + **NC** - b-2Cool® (native type II collagen)	++	+	++	++

CONCLUSION: This study highlights the beneficial effects of an oral combined solution of chondroitin sulphate, glucosamine hydrochloride and hyaluronic acid on joint health. Even better results were obtained when adding b-2Cool® native type II collagen.

3. CLINICAL STUDY: OBSERVATIONAL RETROSPECTIVE STUDY TO EVALUATE POTENTIAL THERAPEUTIC EFFICACY OF NATIVE (UNDENATURED) TYPE II COLLAGEN[12]

Scarpellini M, et al. Biomarkers, type II collagen, glucosamine and chondroitin sulphate in osteoarthritis follow-up: the "Magenta osteoarthritis study". J Orthop Traumatol., 2008, vol. 9, no. 2, pg. 81-87.

OBJECTIVE

To determine the therapeutic efficacy of native type II collagen in combination with glucosamine and chondroitin sulphate.

METHODS

An observational retrospective study, one-year follow-up, on 104 patients with osteoarthritis (nodular hand OA, erosive hand OA (EOA), knee or hip OA) who were treated with glucosamine and chondroitin sulphate (GC) or glucosamine, chondroitin sulphate and collagen type II (GCC).

57 were treated with GCC and 47 with GC. Data was collected at baseline, six and twelve months: patient global assessment (VAS), C-terminal cross-linking telopeptides of collagen types I (uCTX-I) and II (uCTX-II) and radiographs (only at baseline and twelve months).

RESULTS

After six and twelve months of treatment, VAS, uCTX-I and uCTX-II mean values were significantly lower than the baseline. The group that received GCC showed a similar VAS mean value after six and twelve months when compared with the group treated with GC. The uCTX-I (figure 8) and uCTX-II (figure 9) mean levels were lower in the GCC-treated group ($p<0.05$).

Radiological score (figure 10) showed reduced disease progression in hand osteoarthritis after one year of treatment, especially in the GCC group ($p<0.05$).

Figure 8: Urinary C-terminal cross-linking telopeptides of type I collagen in EOA group, GCC vs. GC

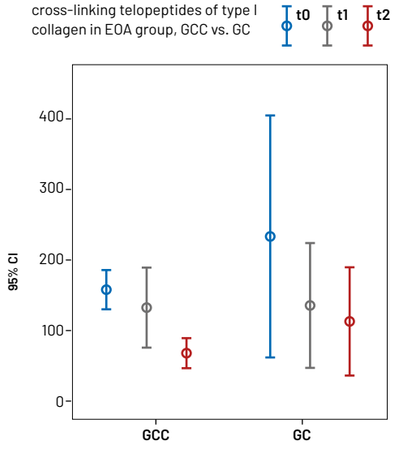


Figure 9: Urinary C-terminal cross-linking telopeptides of type II collagen in hand OA and hand EOA, GCC vs. GC

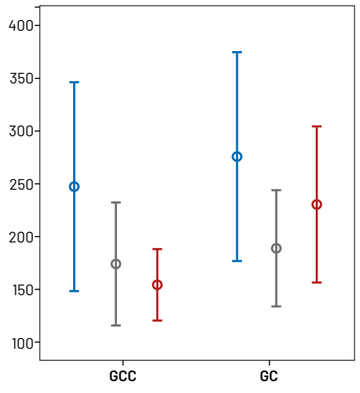
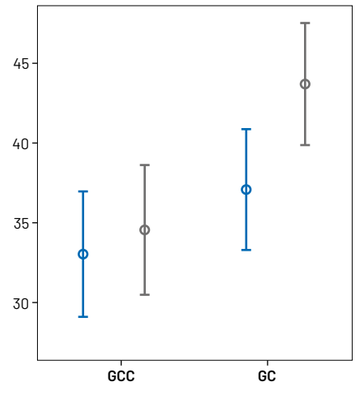


Figure 10: Evolution of radiological score in hand OA and hand EOA, GC vs. GCC



CONCLUSION: The addition of native type II collagen to Glucosamine-Chondroitin (GCC group) further improved the obtained results of the Glucosamine Chondroitin (GC) combination. The GCC-treated group showed better results in reducing collagen destruction and osteoarthritis progression compared to the GC-only group.

4. CLINICAL STUDY: RANDOMISED CONTROLLED STUDY TO ASSESS THE EFFICACY OF NATIVE (UNDENATURED) TYPE II COLLAGEN ON THE SYMPTOMS AND BIOMARKERS OF CARTILAGE DEGRADATION[13]

Bakilan F, et al. Effects of native type II collagen treatment on knee osteoarthritis: a randomised controlled trial. Eurasian J Med., 2016, vol. 48, no. 2, pg. 95-101.

OBJECTIVE

To evaluate the effect of native type II collagen on knee OA when used concomitantly with acetaminophen.

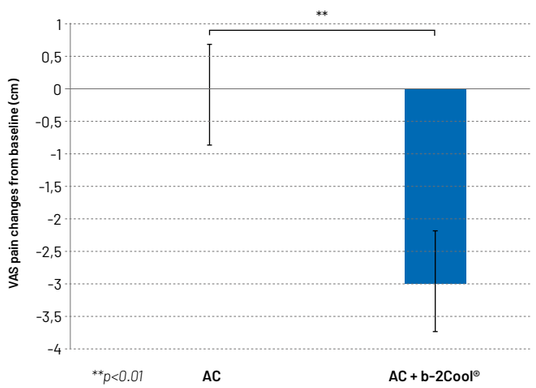
METHODS

39 patients with knee OA were included and randomly distributed into two groups: one treated with 1500 mg/day of acetaminophen (group AC; n=19) and the other treated with 1500 mg/day of acetaminophen plus 40 mg/day of b-2Cool® (group AC+CII; n=20) for three months. Visual Analogue Scale (VAS) for pain at rest and during walking, Western Ontario McMaster (WOMAC) pain, WOMAC function, and Short Form-36 (SF-36) scores, were recorded.

RESULTS

After three months of treatment, significant improvements compared to baseline were reported in pain, function and quality of life and as measured by VAS walking ($p<0.001$), WOMAC pain ($p=0.003$), WOMAC total ($p=0.004$), WOMAC physical function ($p=0.016$) and subscales of SF36 in the AC+CII group. Only some subscales of the SF-36 survey and VAS walking showed improvement in the AC group. Comparisons between the groups revealed a significant difference ($p=0.002$) in VAS walking score in favor of the AC+CII group, when compared to the AC group (figure 11).

Figure 11: VAS pain changes with b-2Cool® supplementation



CONCLUSION: These results suggest that native type II collagen combined with acetaminophen is superior to only acetaminophen in patients with knee osteoarthritis.

NOTES

b 2cool®

NATIVE TYPE II COLLAGEN

ABOUT BIOIBERICA:

Bioiberica is a global Life Science company specialised in the identification, extraction and development of biomolecules of high biological and therapeutic value for the pharmaceutical and nutraceutical industries. This specialisation has positioned Bioiberica as the leading Heparin API manufacturer and a world reference in the research, production and sale of other biologically-derived APIS and ingredients such as Chondroitin Sulphate, Glucosamine, Hyaluronic Acid, Native Type II Collagen or Thyroid. Since 1975, Bioiberica has consolidated its position as an expert in joint health and mobility thanks to a constant commitment to science and research.

These statements have not been evaluated by competent food authorities. This information is only for business-to-business use. The product is not intended to diagnose, treat, cure, or prevent any disease. These statements are not meant to be addressed to final consumers and therefore, Bioiberica assumes no liability for the statements that the producer of the final product may include in its own publicity to consumers.

For more information about Bioiberica's extensive R&D expertise and complete portfolio of naturally-sourced ingredients, visit www.bioiberica.campaign.page/b-2cool

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