FOR EXCLUSIVE USE
BY HEALTH PROFESSIONALS

SCIENTIFIC INFORMATION supporting health benefits of COLPROPUR D® HYDROLYZED COLLAGEN





BENEFICIAL EFFECTS:

- **Helps to prevent and manage diseases and disorders associated with endogenous collagen deterioration:** osteoarthritis, osteoporosis, ligament - muscle - tendon injuries, skin aging, skin wounds, pressure ulcers, fibromyalgia and sarcopenia
- **Helps to improve body composition, sport performance and skin condition**
- **Its collagen bioactive peptides contribute to protect:** immunology, metabolism, cardiovascular and gastrointestinal functions and gut microbiota

Colpropur D®

RECOMMENDED PRODUCT
OAFI
OSTEOARTHRITIS FOUNDATION INTERNATIONAL

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ROLE OF COLLAGEN PROTEIN

Collagen is the main structural protein of the extracellular matrix (ECM) of mammalian connective tissues **(Fig. A)**. Of all the protein constituents in our body, collagen is the most abundant, ubiquitously distributed and multifunctional. It constitutes approximately 30% of the proteins in the human body and is involved in structural and locomotor functions, as well as immunological, cardiovascular, and metabolic ones.

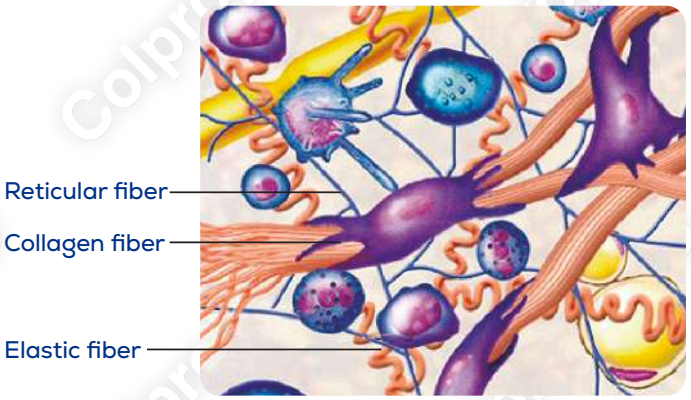


Fig. A. ECM representation

Currently, 29 types of collagen have been discovered. The three major classifications are: type I (bone, tendon and skin), type II (cartilage), and type III (vasculature and skin). Collagen can be found also in other tissues (ocular cornea, scalp, gums and dentin) and organs (liver, kidneys, lungs and heart).

All types of collagen are based on a triple helix configuration made up of three α-chains containing around one thousand amino acids (AAs) each one. The superhelical winding is right-handed and in the opposite direction to the left-handed helix of the α-chains **(Fig. B)**.

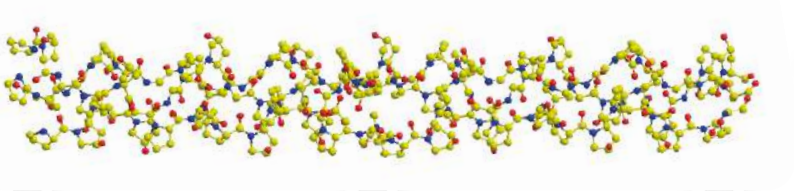


Fig.B. Tropocollagen triple helix

Also, collagen triple helix has the ability to self-assemble forming microfibrils, fibrils and fibers **(Fig.C)**. The geometry and packing of this fibrillar superstructure is specific of each tissue and type of collagen as well as the responsible for the high level of strength, flexibility and other mechanical properties of connective tissues.

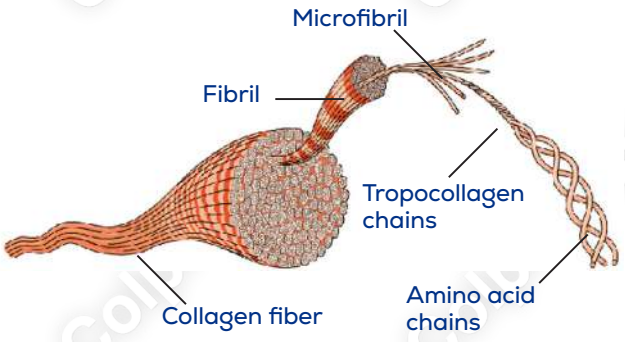


Fig.C. Collagen self-assemble structure

Collagen composition makes it unique among animal proteins due to its high content of glycine, proline and hydroxyproline, three AAs that made up approximately the 50% of collagen amino acid composition. Also, hydroxylated forms of proline and lysine are collagen specific AAs.

Collagen biosynthesis begins inside specific cells (intracellular stage) of each tissue (fibroblasts, osteoblasts, chondrocytes...). The newly synthesized procollagen is secreted to the ECM (extracellular stage) and transformed into tropocollagen ready to self-assemble.

EVOLUTION OF ENDOGENOUS COLLAGEN THROUGH THE LIFE STAGES: CAUSES AND CONSEQUENCES OF ITS LOSS AND DETERIORATION

From early adulthood (25-30 years), collagen-producing cells become less active and collagen production declines by about 1.0%-1.5% a year. This process is accentuated around the age of 45 and, in addition to age, it can be aggravated by certain lifestyle choices like smoking, unhealthy eating, and excessive sun exposure and physical activity, all of them factors that lead to an oxidative stress increase (uncontrolled production of free radicals) that damage endogenous collagen and accelerate aging. Decrease in hormone levels associated with menopause contributes significantly to accelerate collagen deterioration and loss.

Many studies have shown that collagen peptides supplementation stimulates cells to produce more collagen and all other ECM components, delaying, preventing and alleviating connective tissues diseases, injuries and disorders associated with collagen endogenous loss and deterioration like osteoarthritis **(Fig. D)**, ligament and musculotendinous injuries **(Fig. E)**, skin aging **(Fig. F)** and osteoporosis **(Fig. G)**.

OSTEOARTHRITIS

Collagen constitutes about 67% of the dry weight of cartilage. Its loss causes its thinning and weakening, which triggers the osteoarthritic process.

Healthy coxo-femoral joint

Osteoarthritis coxo-femoral joint

Fig. D

LIGAMENT AND MUSCULOTENDINOUS INJURIES

Collagen constitutes about 80% of the dry weight of tendons, ligaments and intramuscular connective tissue (fascia). Its loss favors the appearance of tendon pathologies, sprains, tears and muscle damage.

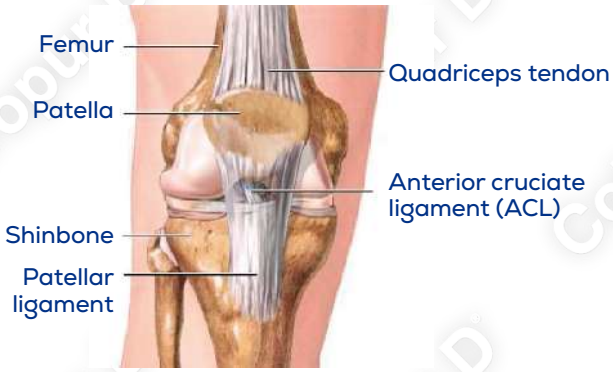


Fig. E

SKIN AGING

Collagen constitutes about 70% of the dry weight of the skin and is concentrated in the dermis. Its loss causes the skin to become thinner and flaccid, and the appearance of wrinkles in areas of frequent flexion.

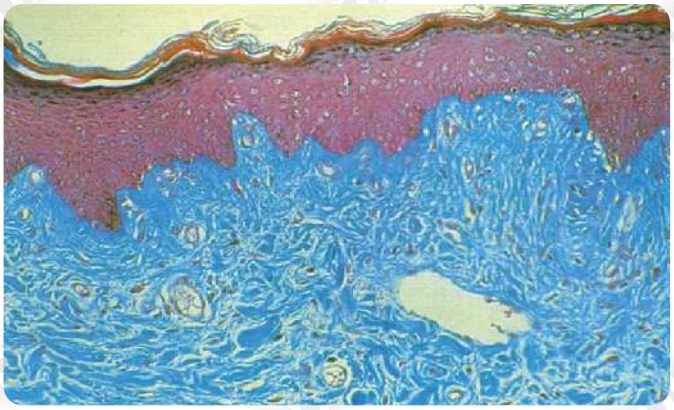


Fig. F. Blue-stained collagen fibers on a cross section of skin

OSTEOPOROSIS

Collagen constitutes 90% of bone organic matrix (35% of bone dry weight). Bone matrix is the support on which calcium salts are fixed (65% of bone dry weight) **(Fig. G)**. When bone collagen deteriorates, calcium salts are released (decalcification) **(Fig. H)**. Even if the diet is supplemented with calcium and vitamin D, it is not enough to mineralize the bone matrix if its regeneration is not stimulated.

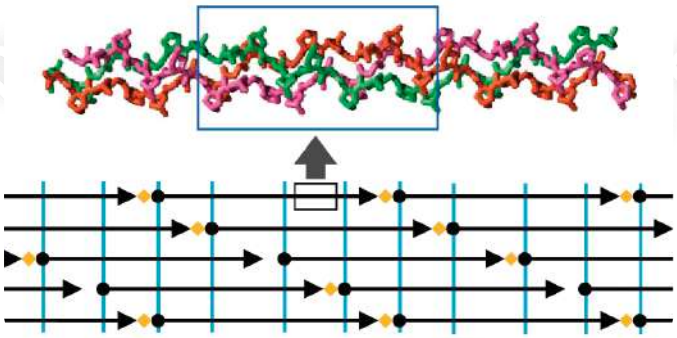
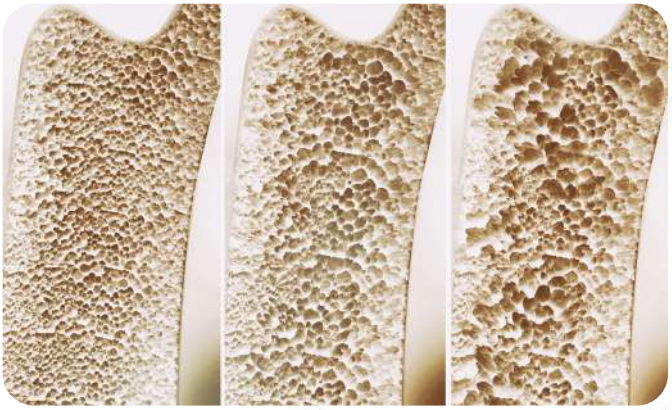


Fig. G. Binding of HAP crystals to collagen of bone matrix



Osteoporosis is the most prevalent metabolic bone disease due to the increase in life expectancy and in diseases associated with adulthood.

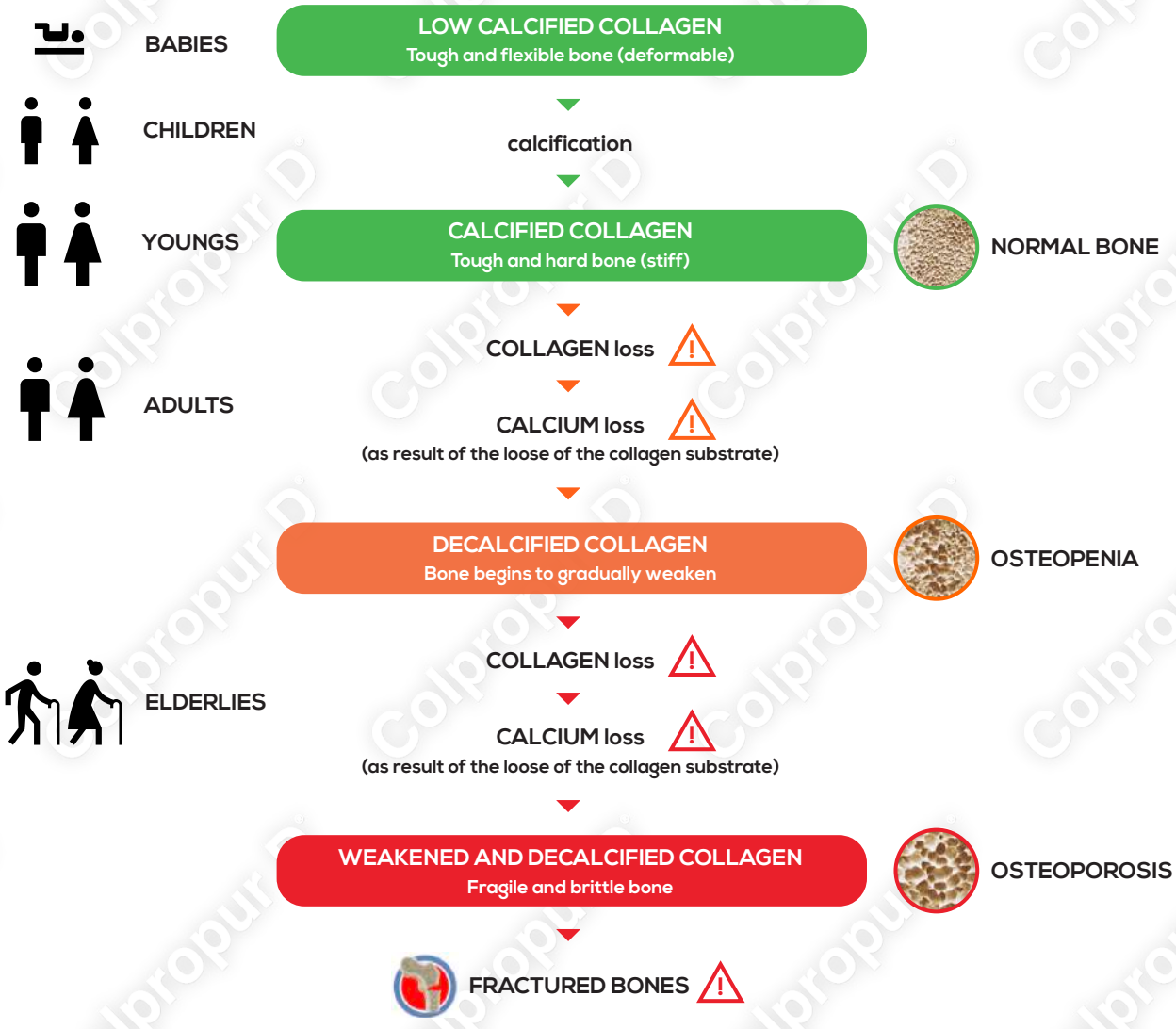


Fig. H. Evolution of bone structure through life stages

HYDROLYZED COLLAGEN

Hydrolyzed collagen (HC) is formed by peptides with an average MW between 3,000 and 5,000 Da. It is obtained from gelatinization and subsequent enzymatic hydrolysis (fragmentation) of native animal collagen. Scientific studies confirm that taken orally, HC is highly bioavailable. In addition, it has been shown that the amino acids (AAs) and peptides derived from its intake act at two levels:

- **Nutritional and basic bioactivities**: specific collagen peptides and AAs contributes effectively to stimulate collagen endogenous biosynthesis and nourish connective tissues, helping to reduce, prevent and slow down their deterioration. Clinical studies show that taking 10 grams of HC daily helps to reduce joint pain due to wear (osteoarthritis), bone mass loss (osteopenia or osteoporosis) and skin aging. HC intake has also shown to be useful for the management of ligament, muscle and tendon injuries, fibromyalgia, sarcopenia and pressure ulcers.

- **Special bioactivities**: collagen peptides act as biological signals (biopeptides) modulating several physiological functions whose compliance is beneficial to relevant health aspects as immunology, metabolism, cardiovascular and gastrointestinal functions, and gut microbiota.

HC beneficial effects and underlying mechanism of action are exposed in more detail through the assessment of scientific studies results.

SCIENTIFIC STUDIES

INTRODUCTION

Hydrolyzed collagen (HC) first studies date back to the 1980s and, since then, they have increased and diversified considerably, especially in the last decade, currently exceeding 300 publications in indexed scientific journals.

It is noteworthy that currently existing studies on HC have been carried out with collagen from a wide variety of tissues and animal species. However, a recent study that has carried out a bioinformatic collagen analysis from 5 different animal sources (bovine, porcine, chicken, trout and salmon) has concluded its interchangeability **[1]**. In fact, slight differences in amino acid (AA) ratios between collagens of different species, tissues and types are too small to produce different effects in the intake of a functional food ingredient intended for a sustained daily consumption.

BIOAVAILABILITY

Studies of bioavailability **[2-6]** show that, after digestion and intestinal absorption, AAs and small peptides are the main derivatives of HC intake that enter bloodstream and reach organs and tissues, where they act nutritionally, supplying basic structural units (AAs), and physiologically, stimulating different biological pathways (bioactive peptides), as the studies in the following sections show.

- An *in vivo* study **[3]** compares absorption of ^{14}C labeled gelatin hydrolysate (GH) to absorption of ^{14}C labeled proline. Results reveal that 95% of the administrated GH is removed from the gut within subsequent 12h to oral administration. Furthermore, a significantly higher and longer-lasting accumulation of labeled GH is observed in cartilage than in other organs and tissues **(Fig. 1)**.

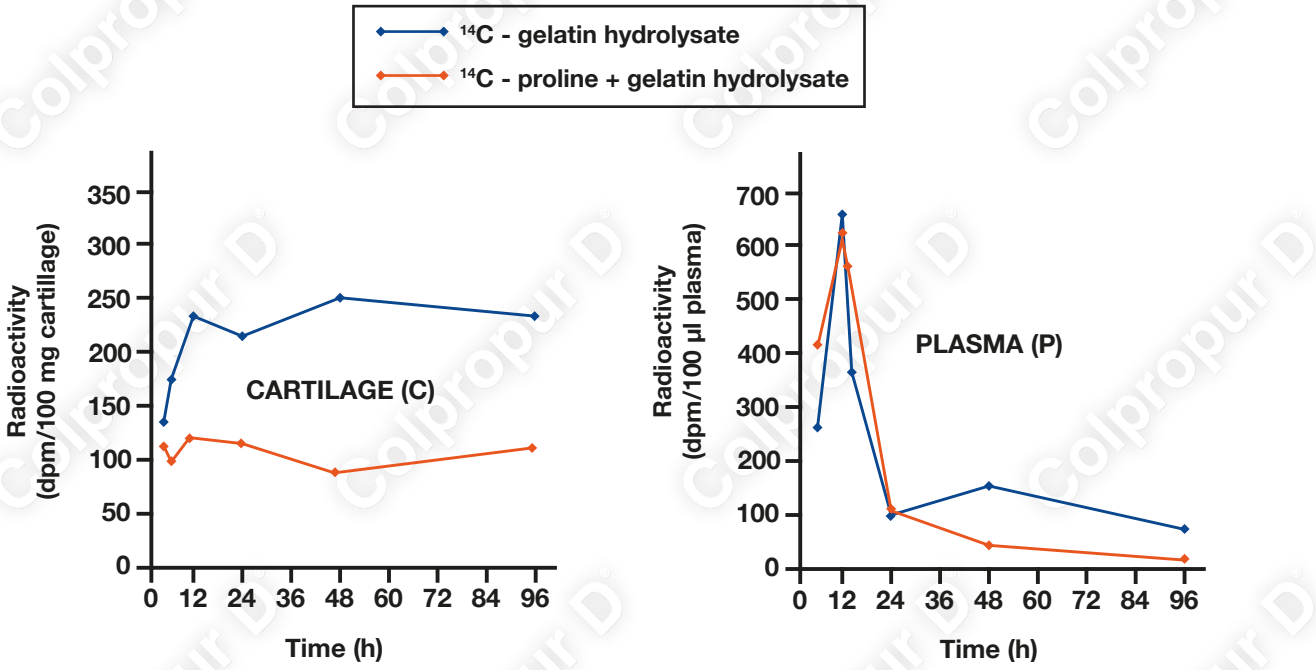


Fig. 1. *dpm: disintegrations per minute.*

- Two studies carried out with **COLPROPUR D®** (under the Colnatur® brand) support its high digestibility and bioavailability. On the one hand, an in vitro study [6] using a dynamic simulator of the human digestive system shows that, 6h after oral administration, 82% of the product has been perfectly digested at the gastric and intestinal level (jejunum and ileum dialysate) and is in optimal conditions to be absorbed and pass into the bloodstream **(Fig. 2)**.

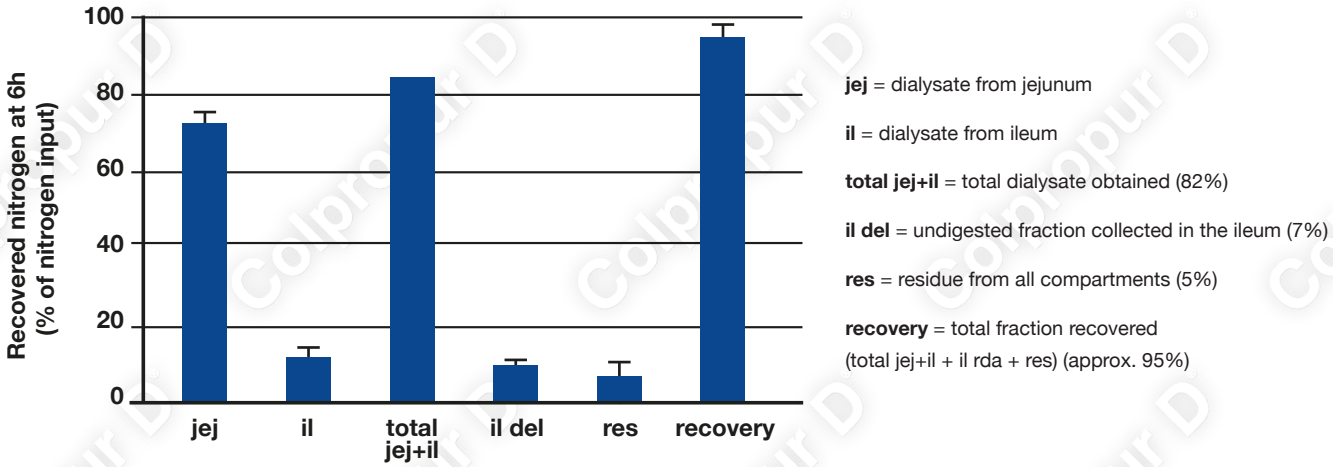


Fig. 2

- On the other hand, a clinical study **[5]** evaluates plasma concentration for the four AAs more representative of HC composition (glycine, proline, hydroxyproline and hydroxylysine) when the product is administrated to healthy adult subjects mixed with water or with yogurt. In all cases, a peak of blood AAs concentration appears only at 1-1.5 h after the ingestion, revealing the high digestibility and bioavailability of **COLPROPUR D®**.

In summary, previous studies show that HC is a protein with high digestibility and bioavailability that provides appreciable amounts of collagen-specific AAs and small bioactive peptides. The former are essential to sustain the constant turnover of connective tissues and, therefore, to maintain their good condition. The latter act as biological signals, stimulating physiological processes whose compliance is beneficial for health and being useful for the prevention and management of disorders and diseases associated with the deterioration of endogenous collagen.

JOINT COMFORT AND OSTEOARTHRITIS (OA)

So far, OA has no cure. The rescue medication commonly used to reduce joint pain (NSAIDs: *non-steroidal anti-inflammatory drugs*) do not stop the progression of OA and their continued use can cause severe adverse side effects. *Symptomatic Slow Action Drugs for Osteoarthritis* (SYSADOAs), as glucosamine and chondroitin, are a slower acting option with fewer adverse side effects. However, these drugs are quite controversial due to their uncertain efficacy and wide variability in the prescription mode.

Within this precarious scenario, a functional food ingredient that provides bioactive peptides and AAs that play a key role in the synthesis of collagen, the main structural protein of cartilage and other joint tissues, emerges as a promising alternative. This is what *in vitro* **[7-9]**, *in vivo* **[10-13]** and clinical studies **[14-22]** confirm. Furthermore, HC is perfectly compatible with NSAIDs and SYSADOAs. But, unlike these, it is possible to take it continuously, so it allows the patient to be protected during the rest periods of those.

In vitro studies

Cartilage integrity and functionality reside in extracellular matrix (ECM) conditions, which are regulated by chondrocytes.

In vitro studies **[7-9]** show that collagen peptides can modulate animal and human chondrocyte activity, stimulating anabolic processes in ECM and, thus, contributing to reduce degenerative changes of cartilage tissue.

- In bovine chondrocytes cultures, it is observed a significant increment of type II collagen biosynthesis when culture medium is supplemented with HC **(Fig. 3B)**. This effect is dose-dependent and does not occur when non-hydrolyzed collagen (i.e. native type II collagen) or collagen-free protein hydrolysate is used **[8]**. In human and porcine chondrocytes cultures, it is observed a significant increment of proteoglycans biosynthesis when culture medium is supplemented with HC **(Fig. 3A) [9]**.

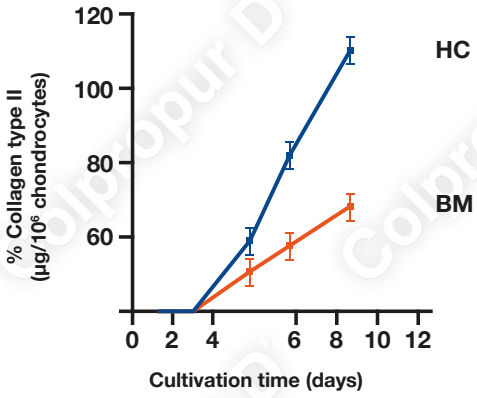


Fig. 3A

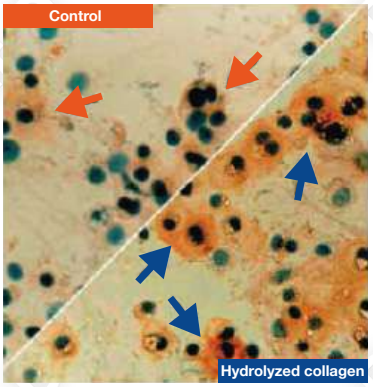


Fig. 3B. Cultures of cartilage tissue, with or without HC. The chondrocytes are dyed blue and the type II collagen synthesised by them, red.

- Furthermore, a study conducted on **COLPROPURD®** (under Colnatur® brand) **[7]**, using its dialysate (digested fraction) to supplement human chondrocyte cultures, shows that the increase in proteoglycan production is dose-dependent and requires a threshold concentration of collagen peptides (1mg/mL) to be triggered **(Fig. 4)**.

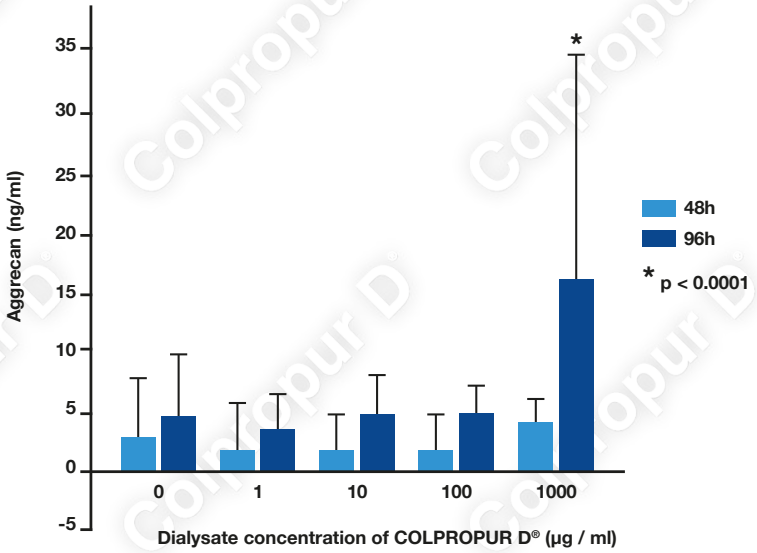


Fig. 4. Synthesis of aggrecans at 48h and 96h in chondrocyte cultures incubated with different concentrations of **COLPROPUR D®** dialysate.

In vivo studies

In OA-induced animal models, results suggest that HC administration increases mobility (less stiffness and lameness in dogs) **[10]**, cartilage area **[11]** and hyaluronic acid and proteoglycan production **[11-13]**, and inhibits mineralization of chondrocytes **[12]**.

CLINICAL STUDIES and META-ANALYSIS

HC clinical studies on joint health target two different population groups: those who are diagnosed with OA **[14-16]** and those who suffer from joint pain induced by physical activity (athletes) without associated pathology **[17-19]**. Some reviews **[20, 21]** and a meta-analysis **[22]** are also available. In all cases, good tolerance and safety of nutritional supplements based on hydrolyzed collagen have been reported.

a) OA patients

Several randomized placebo-controlled trials (RCTs) **[14-16]** are conducted with moderate to severe OA-diagnosed patients. The group who receives a HC daily dose of 10g for 13 to 48 weeks experiences different levels of improvement in pain and stiffness compared to placebo group, assessed by WOMAC index (*Western Ontario McMaster Universities*) and VAS (*Visual Analogue Scale*). These patients also report an improvement in quality of life, assessed by SF-36 Questionnaire (*36-item Short-Form General Health Survey*).

- A RCT **[14]** is conducted with 250 patients diagnosed with knee OA (Kellgren-Lawrence (KL) grades I-III) who take 10 g of **COLPROPUR D®** (under Colnatur® brand) or placebo daily for 6 months. According to VAS scores, the proportion of patients experiencing pain reduction (a decline in VAS ≥ 30 mm) is significantly higher in the treatment group (75%) than in the placebo group (53%) (p = 0.001) **(Fig.5)**.

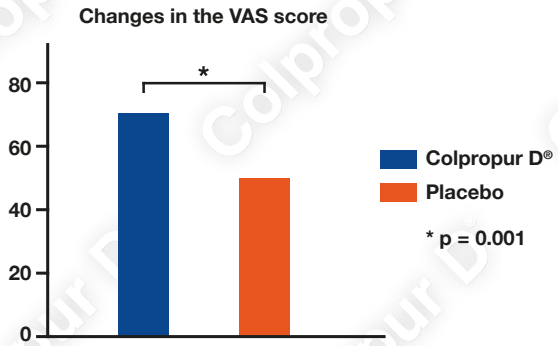


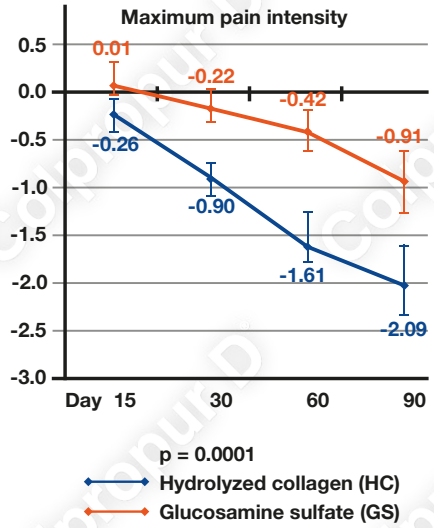
Fig. 5. % of patients with a decrease in knee pain of at least 30 mm on the VAS.

Furthermore, the reduction in pain intensity is significantly higher in patients of the treatment group than in patients of the placebo group (p = 0.024) and even more in the subgroup of patients affected by advance OA (p = 0.015) and in the subgroup of patients with poor intake of animal protein (p = 0.010) **(Fig.6)**.

VAS (Visual Analogical Scale)	% of pain reduction in the HC group compared to the placebo group	p
Total patiens who finished the study	13.86	0.024
Patients with advanced ostheoarthritis	19.25	0.015
Patients with mid-low ingestion of animal protein	34.89	0.010
Patients with advanced osteoarthritis and mid-low ingestion of animal protein	41.59	0.013

Fig. 6

- Other RCT **[16]** compares the efficacy of daily intake of 10g of HC with 1.5g of glucosamine sulfate (GS) for 90 days using quadruple VAS and WOMAC index to assess pain level and SF-36 Questionnaire to assess quality of life. HC demonstrates better clinical efficacy than GS, with significant improvement in pain scores, functional joint status and better quality of life **(Fig.7)**.



Analgesic activity of GS and HC on the quadruple VAS	GS (Glucosamine sulphate)		HC (Hydrolyzed collagen)	
VAS decrease*	No. of participants	%	N°	%
Clear improvement (> 20mm)	17	37.0	32	68.1
Moderate improvement (between 10 and 20mm)	14	30.4	8	17.0
No improvement (< 10mm)	15	32.6	7	14.9
Overall	46	100.0	47	100.0

(*) Differences between two groups with statistical significance: $p < 0.05$

Fig. 7

- Finally, a meta-analysis of 5 RCTs **[22]** shows a global improvement in OA symptoms following oral administration of collagen-based supplements, reflected by the decrease of both total WOMAC index and VAS score. The study mentions that hydrolyzed collagen exhibits stronger therapeutic benefits for the management of OA than undernatured type II collagen probably due to its higher absorption.

b) Athletes

- An observational study **[17]** and a RCT **[18]** based on VAS scores **(Fig. 8)** show an improvement in joint pain in lower and upper extremities, especially significant in the knee, in athletes after taking 10g of HC daily for 3 and 6 months respectively.

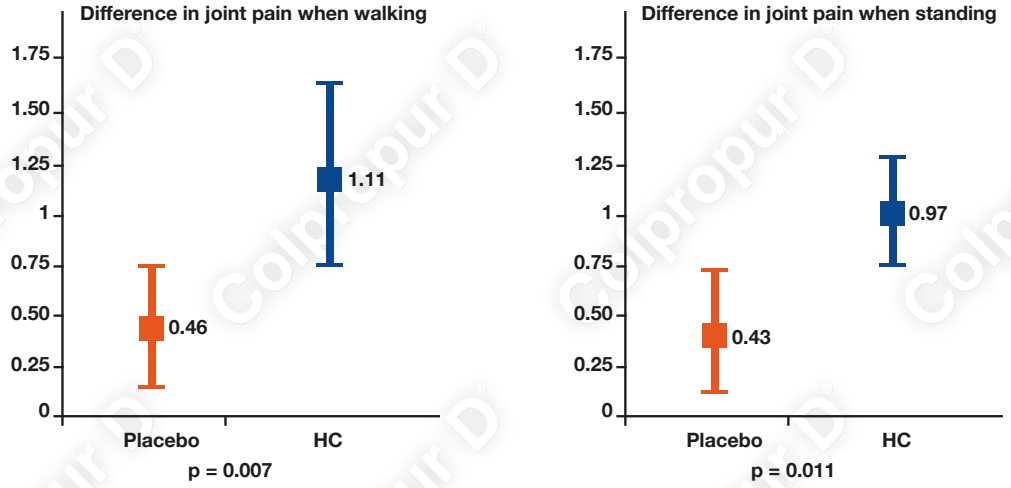


Fig. 8. Change of VAS between the initial visit and the last visit. The higher the number, the higher the reduction of pain.

- In another study **[19]**, members of a men's mountain bike team and a women's basketball team take a daily dose of 10g of HC plus B vitamins and magnesium for 6 months. Ultrasound scan of the scapula-humeral and femora-tibial joints show significant positive changes in cartilage biometric measures compatible with an increase in articular cartilage mass in the treated group. In contrast, in the control group no improve is observed.

In summary, previous studies show that HC intake promotes the good condition and functionality of joint tissues, stimulating its formation (especially for cartilage) and, consequently, helping to prevent, slow down and reduce joint wear and pain. These effects are especially important for people with intense physical activity (athletes) and in the elderly. In addition, continuous intake of HC is useful for the management of OA.

BONE LOSS AND OSTEOPOROSIS (OP)

As in the case of OA, there is currently no cure for OP. Conventional drugs are based on inhibiting bone resorption, but their efficacy is limited and their prolonged use can cause adverse side effects such as suppression of bone formation, favoring osteonecrosis.

Nutritional strategies are traditionally based on supplementing the diet with calcium and vitamin D. However, there is much controversy regarding its efficacy and proper mode of use. Furthermore, this supplementation only targets the mineral part of the bone. However, it is essential to maintain the good condition of the bone organic matrix (35% of bone dry weight), composed mainly of collagen fibers (90% type I collagen), to which calcium salts are adhered (65% of bone dry weight).

The loss and deterioration of endogenous collagen due to age and estrogen level decrease in menopause cause the weakening of bone matrix. Consequently, calcium salts are released (decalcification), which increases the risk of bone fracture. Calcium and vitamin D supplementation is not enough at this time to prevent decalcification, since it is necessary previously to reinforce the bone organic matrix.

In vitro studies

- These studies show that collagen peptides promote osteoblast proliferation and differentiation, and stimulate their activity, producing an increase in collagen synthesis and subsequent mineralization **[23-25]**.

- In addition, some *in vitro* studies **[26,27]** suggest that the mineral-binding capacity of collagen peptides can be used to form peptide-mineral complexes that increase calcium bioavailability while feeding both the organic and the mineral part of bone. This is the case of **PHOSCOLLAGEN® (PHC)**, a peptide-mineral complex formed by **COLPROPUR D®** HC and hydroxyapatite (HAP, the bone-specific calcium phosphate salt), both from fresh bovine bones, in which the micronized HAP is stabilized in a matrix of collagen peptides, forming a homogeneous ingredient that is not a simple mixture, but an uniform compound.

- In fact, in an *in vitro* study **[27]** carried out with human osteoblast cultures treated with **PHOSCOLLAGEN®**, after simulating its gastrointestinal digestion and absorption, it is observed a significant increase in the proliferation of pre-osteoblasts **(Fig. 9)** and in the production of bone biomarker gene expression associated with the osteogenic activity of mature osteoblasts **(Fig. 10)**. These results suggest that **PHOSCOLLAGEN®** is a functional food ingredient useful for promoting bone formation.

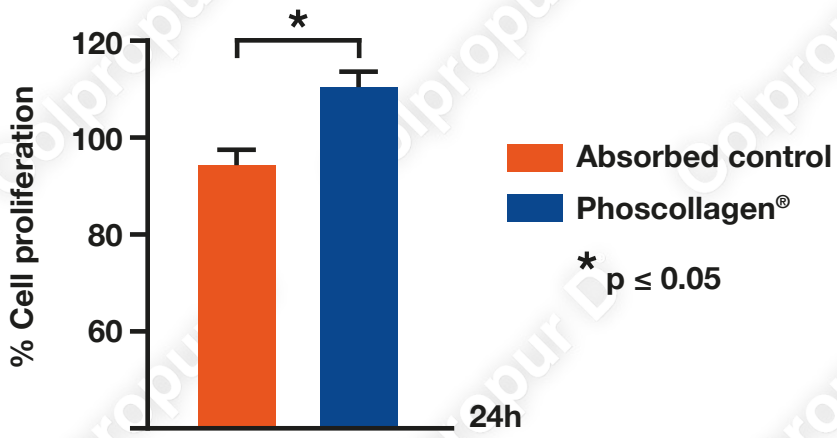


Fig. 9

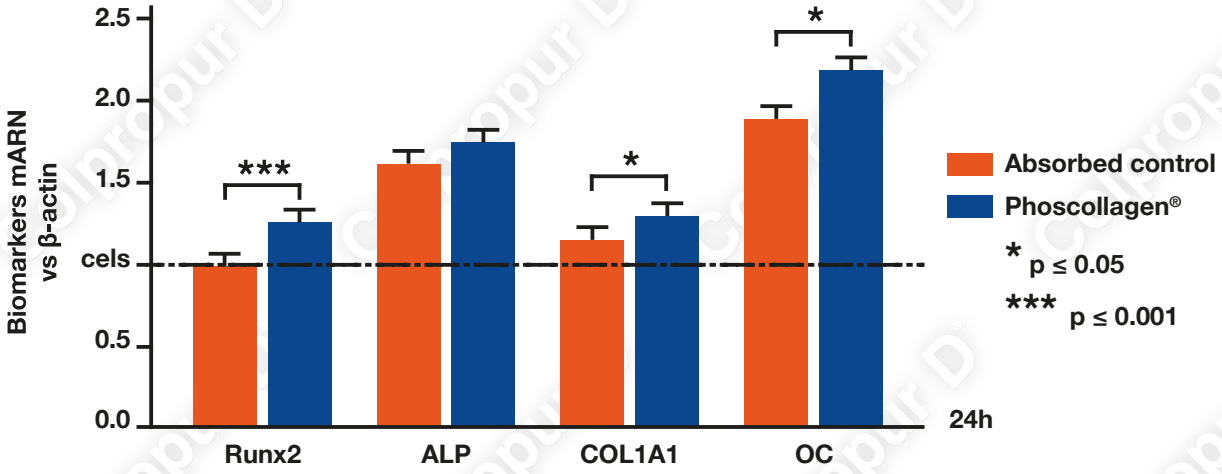


Fig. 10. Treatment of mature osteoblast withabsorbed **PHC** fraction produces an increment of following bone biomarker gene expression: Runx2 (specific transcription factor forosteogenesis), ALP (alkaline phosphatase), COL1A1 (gene that encodes α1 chains of type-1 pro-collagen) and OC (osteocalcina).

In vivo studies

These studies suggest that collagen peptides can increase BMD (bone mineral density) and improve trabecular microarchitecture in postmenopausal **[28-30]**, chronologically aged **[31]**, growing **[32,33]** and deprived of mechanical stimuli **[34]** animal models.

CLINICAL STUDIES

These studies focus on two population groups for which bone metabolism is especially important:

a) Postmenopausal women

- A RCT **[35]** is conducted with 108 postmenopausal osteoporotic women for 6 months. All of them receive intramuscular calcitonin (CAL) twice a week and a group of 49 also receives 10g of HC daily. The CAL + HC group experiences a significant higher decrease in urine bone resorption markers than the CAL group ($p \leq 0.05$). Furthermore, the significant reduced values of urine bone resorption markers persist 3 months after finishing the HC intake. Study authors conclude that oral administration of HC enhanced and prolonged notably the effect of calcitonin **(Fig. 11)**.

	CAL			CAL + HC		
Time (months)	0	6	9	0	6	9
UPD[1] (% of initial value)	100.0% (*)	63.5%	60.8% (**)	100.0% (*)	47.8%	45.5% (**)
UDPD[2] (% of initial value)	100.0% (*)	77.4%	58.1% (**)	100.0% (*)	47.0%	48.2% (**)

[1] Urinary excretion of pyridinoline

[2] Urinary excretion of deoxypyridinoline

(*) initial values are adjusted to 100 to facilitate comparison of the % of biomarkers that remain in urine

(**) after 3 months of finishing treatment

Fig. 11

- In two RCTs conducted with postmenopausal osteopenic women who are supplemented with vitamin D_3 and calcium, the group that receives a calcium-collagen chelate supplement for 3 months **[36]** or for 6 months **[37]** experiences, compared to the group that receives placebo, a significant improvement in total BMD and in bone formation/ resorption markers ratio (BAP/TRAP5b) in serum. Study authors conclude that the intake of calcium-collagen chelate reduces bone loss due to the fact that it stimulates the formation versus the resorption in the process of bone turnover, which does not occur to the same extent if the supplementation only consists of calcium and vitamin D_3.

b) Children and pre-pubertal children

Bones continue to develop until the age of 35 years and it's known that optimal bone peak mass must be attained to prevent bone disease in later life.

- In a RCT **[38]** conducted with 60 children aged 6 to 11 of both genders and a low-calcium diet, it is observed that the group that receives a calcium and HC supplement for 4 months experiences, compared to the groups that receive placebo or only HC, a significant increase in bone formation markers (ALP) and a significant decrease in bone resorption markers (TRAP and CTX) in serum. Study authors conclude that the intake of HC during growth stimulates bone formation as long as calcium intake is adequate and probably prevents the appearance of bone diseases in advanced ages.

In summary, previous studies show that HC supplementation significantly improves the results of calcium and vitamin D supplementation, thanks to the fact that HC intake stimulates bone formation while improving calcium absorption. This effect increases when used in form of hydrolyzed collagen-hydroxyapatite complex (PHOSCOLLAGEN®).

Also, HC is very suitable as coadjuvant in osteopenia and OP treatment with antiresorptive drugs, since its anabolic action complements the antiresorptive action, reduces the risk of side effects (osteonecrosis) and protects patients during rest periods.

SKIN AGING AND SKIN DAMAGE

Collagen is the main component of the dermis. As it happens with bone collagen, skin collagen content decreases and deteriorates with aging. Indeed, dermis organic matrix comprises about 80% collagen, mainly type I, and bone organic matrix comprises about 90% collagen, mainly type I also. Furthermore, several studies with elderly women suggest that skin thinning and bone loss are correlated **[39]**.

Preclinical studies

In vitro studies **[40-42]** show that collagen peptides stimulate fibroblasts proliferation and activity, increasing the biosynthesis of type I collagen, elastin and hyaluronic acid. *In vivo* studies show that HC administration can attenuate skin aging **[43]** and photoaging **[44]**, and improve the closure of skin wounds produced by different causes as burns, surgical excisions, pressure ulcers and diabetic wounds **[45-48].**

CLINICAL STUDIES and META-ANALYSIS

These studies focus on two aspects: attenuation and delay of skin aging and healing of wounds of different types.

a) Antiaging

Studies reviews **[49-51]** and a meta-analysis **[52]** show that HC intake can improve skin properties, especially moisture, elasticity, density and firmness, and reduce wrinkling and roughness.

- A RCT **[53]** is conducted with 50 healthy postmenopausal women who take 10 g of **COLPROPUR D®** (under Colnatur® brand) or placebo daily for 3 months. When comparing the microscope images of the silicone molds made of the periocular area before and after treatment, a clear improvement is observed **(Fig. 12)**.

Fig. 12

Also, there is a clear improvement in the total number (p = 0.033) and length, surface and depth of wrinkles in the treated group compared to placebo group **(Fig. 13)**.

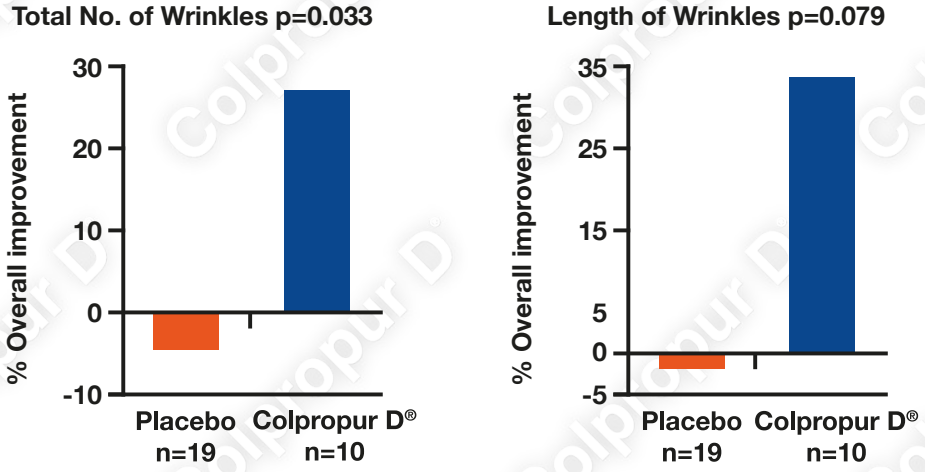
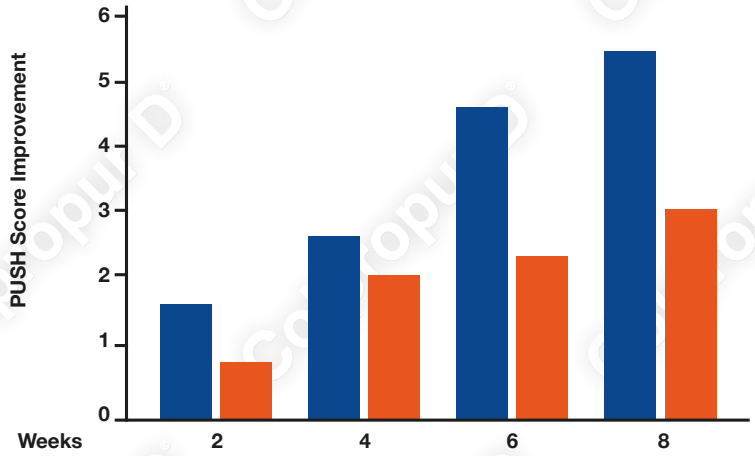


Fig. 13

b) Wound healing

- Several RCTs **[54-56]** show that the rate of pressure ulcers (UPP) healing increases significantly if daily HC supplement is added to standard therapy **(Fig. 14)**.



Weeks	2	4	6	8
Treatment	1.52	2.56	4.56	5.56
Control	0.77	2.11	2.29	2.85

Fig. 14. Pressure Ulcer Scale for Healing (PUSH) scores in long-term care residents receiving standard care plus HC supplement (treatment group) versus residents receiving standard care plus placebo (control group,). Scores are approximately 96% greater in the TG than in the CG **[54].**

- Also, in a RCT **[57]** conducted with 39 hospitalized older adults, the group taking 10g of HC daily for 8 weeks shows a significant increase in stratum corneum hydration and skin elasticity, suggesting that collagen peptides can reduce skin vulnerability in older adults and, in consequence, prevent chronic wounds.

It is noteworthy that to achieve some type of improvement in skin properties, daily doses of 5g-10g of HC are mostly used. However, in the case of UPP in older adults, daily doses of 10-15g of HC are used. Between improving some skin properties and accelerating wound healing, 10g of HC seems to be an adequate daily dose to slow down aging and photoaging, and attenuate and prevent its signs on the skin.

In summary, previous studies show that HC intake promotes skin health acting from different levels: it improves its properties, helps to reduce intrinsic aging and photoaging, protects the epidermal barrier function from external aggressions (UVB radiation), and contributes to accelerate wound healing and prevent the formation of chronic wounds in the elderly.

IMMUNE SYSTEM

Immune competence decreases with age, stress, and malnutrition. There is evidence that some nutrients are capable of influencing immune response and inflammatory processes, being considered as immunonutrients. Supplements based on immunonutrients play important and complementary roles in supporting the immune system.

Preclinical studies

In vitro and *in vitro & in vivo* studies **[58-61]** show that collagen peptides exert health-promoting bioactivity on immune cells and on other cells involved in immune response and inflammatory conditions by modulating cytokines production. Also, it has been observed that collagen peptides exert antiproliferative and cytotoxic activity in human colon cancer (Caco-2), liver cancer (HepG2), breast carcinoma (MCF-7) and glioma (U87) cell lines **[62-64]**.

- An *in vitro* study **[65]** is carried out with human macrophages and T lymphocytes whose cultures are treated with two different **COLPROPUR D®** fractions: one from simulating its ingestion and gastrointestinal digestion (the bioaccesible or digested fraction, DF, equivalent *in vivo* to that in contact with gut immune cells) and other from simulating the absorption process of DF through the intestinal wall (the bioavailable or absorbed fraction, AF, equivalent *in vivo* to that in contact with blood immune cells). Using cytokine mRNA expressions as biomarkers, results show remarkable differences between **COLPROPUR D®** fractions and controls (from carrying out identical processes of digestion and absorption, but in the absence of **COLPROPUR D®**). The main findings are as follows:

  1. An immunostimulating effect on macrophages (M0) in contact with both **COLPROPUR D®** DF (5mg/mL) **(Fig.15A)** and **COLPROPUR D®** AF (625µg/mL) **(Fig.15B)**, which does not occur when macrophages have been previously activated (M1), suggests that **COLPROPUR D®** could exert an alert state without exacerbation of the immune response in case of pathogen appearance.

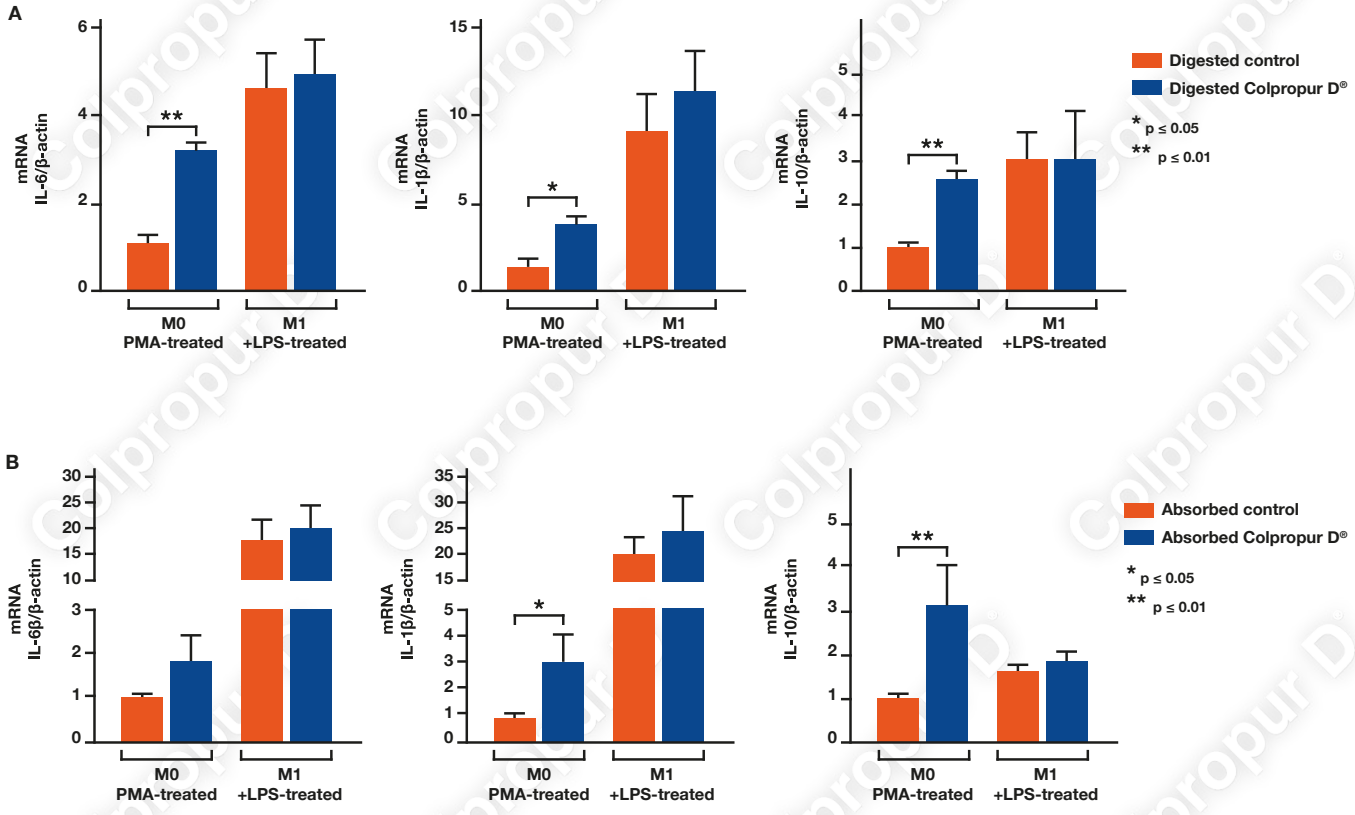


Fig. 15. Significant increase in cytokine expressions are produced by M0 macrophages, but not by M1 macrophages, in conctact with: **A) COLPROPUR D®** DF (5mg/mL) and **B) COLPROPUR D®** AF (625µg/mL).

2. Macrophages and T lymphocytes cell viability at maximum concentration (1/2 dilution) is much higher in contact with **COLPROPUR D®** DF (5mg/mL) than with digested control **(Fig.16).** In addition, T lymphocytes proliferation is significantly higher after 24h (and more after 48h) of finishing treatment with **COLPROPUR D®** AF (625µg/mL) than with absorbed control **(Fig.17)**. These results suggest that **COLPROPUR D®** could exert an immunoprotective effect on gut and blood immune cells respectively.

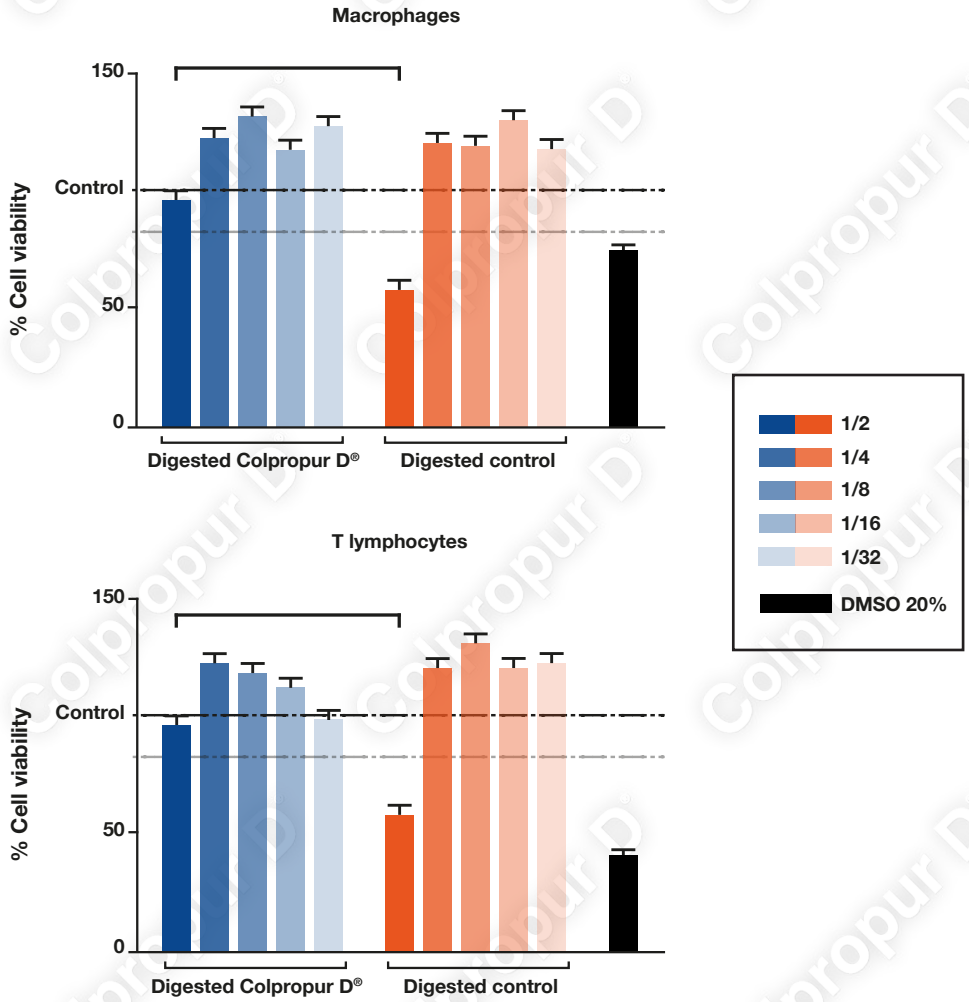


Fig. 16 At maximum concentration (leftmost column of each set: 1/2 dilution), cell viability of macrophages and T lymphocytes is significantly higher in contact with **COLPROPUR D®** DF (5mg/mL) than with respective digested controls. DMSO: dimethyl sulphoxide.

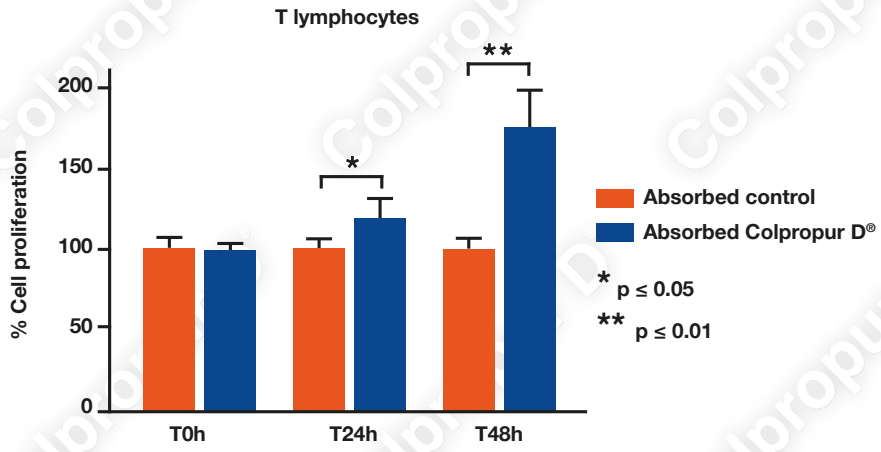


Fig. 17. 24h after finishing treatment with **COLPROPUR D® AF** (625µg/mL), it is observed a significant increase in T lymphocytes proliferation, even more significant 48h after.

3. A significant increase in IL-10 cytokine expression is produced by macrophages (M0) in contact with **COLPROPUR D®** DF and AF **(Fig.18)** and by T lymphocytes in contact with **COLPROPUR D®** DF **(Fig.19)**. IL-10 is an anti-inflammatory cytokine that contributes to restore normality when the inflammatory process has fulfilled its function. These results suggest that **COLPROPUR D®** could exert a systemic (in blood) and local (in gut) anti-inflammatory effect.

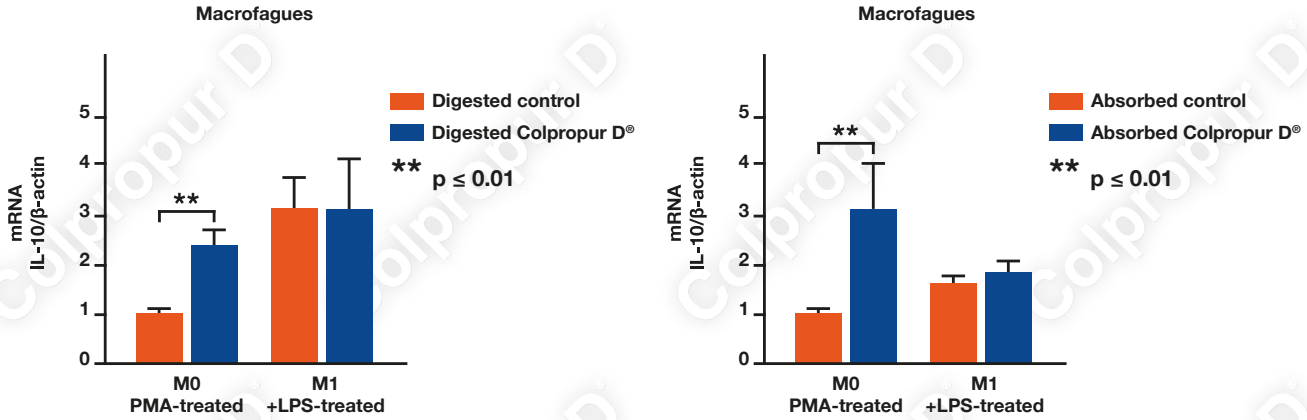


Fig. 18. Significant increase in IL-10 cytokine expression is produced by M0 macrophages in contact with **COLPROPUR D® DF** (2.5mg/mL) and **AF** (625µg/mL).

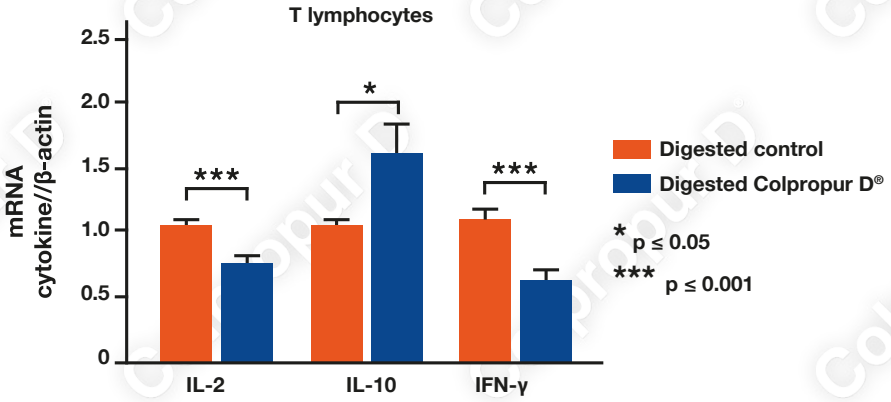


Fig. 19. Significant increase in anti-inflammatory IL-10 cytokine expression and decrease in pro-inflammatory IL-2 and IFN-γ cytokine expressions are produced by T lymphocytes in contact with **COLPROPUR D® DF** (2.5mg/mL).

These results suggest that **COLPROPUR D®** exerts an immunomodulatory activity that can improve immune functions, acting as immunonutrient.

- Other *in vivo* studies show that the administration of HC can improve immune capacity in unfavorable health conditions such as: allergies and inflammatory diseases **[58]**, weightlessness-induced immunosuppression **[66]**, glucocorticoid-induced immunosuppression **[67]**, oxidative damage-induced immunosuppression **[68]** and chronic kidney disease **[61]**. Also, it has been observed that HC administration increases life span and decreases spontaneous tumor incidence in a rat model **[69]**.

- In addition, *in vivo* studies show that HC administration can be useful for improving some autoimmune diseases such as: rheumatoid arthritis by inducing oral tolerance **[70.71]**, inflammatory bowel disease by modulating gut microbiota and exerting local and systemic anti-inflammatory effect **[60.72,73]**, and asthma and other diseases related with airway inflammation **[74]**.

CLINICAL STUDY

A RCT **[75]** is carried out with 50 healthy Japanese men and women aged between 39 and 53 years with daily tiredness and fatigue, and with a relatively low SIV (*Scoring of Immunological Vigor*). Participants take 10g of collagen peptides (treated group) or placebo daily for 8 weeks. The main findings are as follows:

1. A significant improvement in SIV scores of the treated group compared to the placebo group ($p < 0.030$), and even more significant within the treated group ($p < 0.002$) before and after treatment period.
2. T-cell-related parameters (numbers of T cells, memory T cells and CD8+CD28+T cells) improve significantly within the treated group ($p < 0.05$).
3. The number of $CD8^{+}CD28^{+}$T cells increases significantly in the treated group ($p < 0.039$), but not in the placebo group. This parameter represents immunological age and decreases with age. Its increase after taking **10g of collagen peptides daily for 8 weeks is equivalent to a decrease of 1.8 years in immunological age**.
4. A significant improvement in diarrhea and appetite are observed only in the treated group, probably associated to T cells activity modulation exerted by collagen peptides.

Changes in immunological parameters within the treated group after 8 weeks of daily oral intake of 10 g of hydrolyzed collagen

Item	Unit	Placebo	FCP	P value
Score of immunological vigor	–	15.6 ± 1.8	16.2 ± 1.6	0.030
T lymphocyte age	year	50.4 ± 5.8	48.6 ± 10.0	0.121
Neutrophil	/μL	3254.8 ± 815.4	3528.7 ± 1605.6	0.315
Lymphocyte	/μL	1521.8 ± 289.9	1624.4 ± 391.4	0.110
T cell	/μL	1047.6 ± 216.8	1143.5 ± 295.0	0.079
CD4 + T cell	–	681.8 ± 188.9	746.7 ± 234.3	0.161
CD8 + T cell	/μL	389.4 ± 131.1	439.7 ± 164.5	0.148
CD4/CD8 T cell ratio	/μL	2.0 ± 1.1	1.8 ± 0.7	0.514
Naive T cell	–	255.0 ± 128.4	278.4 ± 118.2	0.277
Memory T cell	/μL	426.7 ± 118.9	468.4 ± 152.0	0.197
Naive/memory T cell ratio	/μL	0.6 ± 0.3	0.6 ± 0.3	0.911
CD8 + CD28 + T cell	/μL	230.4 ± 67.8	271.8 ± 138.5	0.911
B cell	/μL	225.5 ± 102.1	241.5 ± 93.0	0.519
NK cell	/μL	195.4 ± 103.5	187.4 ± 112.8	0.765

In summary, previous studies show that HC can act as immunonutrient, modulating immune and inflammatory responses. In fact, HC intake can improve immune status, especially adaptive immunity, and subjective symptoms. These effects could be particularly beneficial for elderly people or those suffering from adverse health conditions that decrease immune competence.

BODY COMPOSITION, LIPID METABOLISM AND SARCOPENIA

Sarcopenia is mainly characterized by the loss of muscle mass associated with age. It is often accompanied by an increase in body fat, a loss of bone mass, and a decrease in strength and functional status.

Adequate dietary protein is essential to maintain lean body mass. Furthermore, there is increasing evidence that aging may be associated with a higher need for dietary protein **[83]**. However, many elderly people consume a relatively low-protein diet. In this context, high quality, low-fat protein supplements have been shown to reduce complications and decrease mortality.

Preclinical studies

Collagen peptides have exhibited lipid-lowering effects *in vitro*, modulating pancreatic lipase **[76]** and adipocyte activity **[77]**. *In vivo*, HC administration in obesity animal model reduces body weight gain **[78]**, fat accumulation and plasma and hepatic triglyceride, and total cholesterol levels **[79,80]**.

CLINICAL STUDIES

- An RCT **[81]** conducted with healthy overweight adults concludes that collagen peptides are useful for reducing body fat. Furthermore, if HC supplementation is added to resistance exercise training (RET), the increase in fat-free mass (FFM) and hand-grip strength, and the decrease in fat mass (FM) are significantly greater than when RET is performed alone, especially in elderly sarcopenic men, and to a lesser extent in pre-menopausal women and recreationally active men **[82]**.
- Dairy proteins result in a greater increase in plasma leucine and EAA (essential amino acids), which seems to facilitate muscle protein synthesis, and HC results in a greater increase in plasma glycine, proline and hydroxyproline, which seems to facilitate the synthesis of intramuscular connective tissue collagen. Furthermore, HC has specific properties that can promote muscle anabolism, especially when combined with physical exercise: **1.** HC provides significant amounts of glycine and arginine, which are both known to be key substrates in the synthesis of creatine, an immediate and direct vector to transport ATP and provide energy to muscle myofibrils. **2.** Collagen peptides bioactivity has shown to influence microcirculation positively (see **[100]** further). This fact increases the delivery of amino acids in comparison to other protein sources and makes the presence of HC recommended in daily protein intake when the objective is to increase lean tissue mass and strength (athletes and mature or old adults with frailty or sarcopenia). **3.** Collagen peptides bioactivity has shown to reduce joint pain and stiffness, and increase joint comfort and functionality, improving physical exercise performance.
- In addition, some studies **[83-85]** show that it is easy and healthier to achieve the optimal combination between collagen and common EAA-rich animal proteins to obtain the proper balance between EAAs and non-EAAs that allows to meet body metabolic needs and take advantage of functional collagen peptides at the same time, thus promoting better maintenance of the musculoskeletal system throughout life and in old age.
- Finally, an observational study **[86]** carried out in runners of both sexes who take daily **COLPROPUR D®** (10g included in the daily dose of Colnatur Sport®) for 16 weeks shows that a resistance training together with the daily intake of **COLPROPUR D®** hydrolyzed collagen enriched with vitamins and minerals improves functionality and performance of the runner in long-distance tests thanks to its beneficial effects on muscles and joints conditions.

In summary, previous studies show that HC contributes effectively to balance protein intake by providing significant amounts of the AAs necessary for collagen biosynthesis and also bioactive peptides that enhance lipid metabolism. These effects promote the good condition and functionality of the musculoskeletal system, as well as the results of physical training and the process of improving body composition, especially important for athletes and people suffering from sarcopenia or physical fragility.

FIBROMYALGIA SYNDROME (FMS)

- Studies **[87]** show that FMS patients have significantly less collagen and less degree of collagen cross-linking in intramuscular connective tissue. This could predispose to suffer muscle micro-injuries, causing the chronic musculoskeletal pain characteristic of tender points in FMS.

- In an observational study **[88]**, 30 participants diagnosed with FMS for at least 2 years and aged between 31 and 75 years take a tablespoon of HC supplement daily for 90 days. Since FMS treatment is based almost entirely on the patient's symptomatic complaints, this study uses numerical scores for patient responses to 11 characteristic FMS symptoms.

One man and nineteen women completed the study. Results show a notable average decrease in general pain and also in temporomandibular dysfunction (TMD). Other improvements occur in sleep quality, irritable bowel syndrome, chronic headaches and cognition/memory **(Fig. 20)**. In fact, after trying many other modalities of treatment, the skepticism of participants in this study makes unlikely that the reported improvements are due to an expectancy effect.

	Start	30 days	60 days	90 days	Average variation	Results
Level of pain (scale 0-10)	6.8	5.8	4.85	5.1	-1.70	25% reduction
Fatigue	50%	48.75%	51.25%	52.5%	-2.50%	2.5% reduction
Total sleep	7.45	6.9	7.4	7.1	-0.35	0.5% decrease hours
Uninterrupted sleep	3.35	3.95	4.1	4.2	-0.85	25% deeper sleep
Intestinal irritability	5.8	5.25	4.25	3.7	-2.10	36% improvement
Chronic migraine	5.9	4.95	4.5	3.9	-2.00	34% improvement
Temporomandibular disorders (muscles and ligaments)	3.75	3.65	2.5	2.3	-1.45	39% improvement
Morning stiffness	8.25	6.45	5.9	5.6	-2.65	32% improvement
Memory loss	6.4	5.0	4.4	4.15	-2.25	35% improvement
Feeling of numbness/tingling	6.95	4.6	3.7	3.9	-3.05	44% improvement
Feeling of extreme swelling	5.0	4.1	3.6	3.7	-1.30	26% improvement

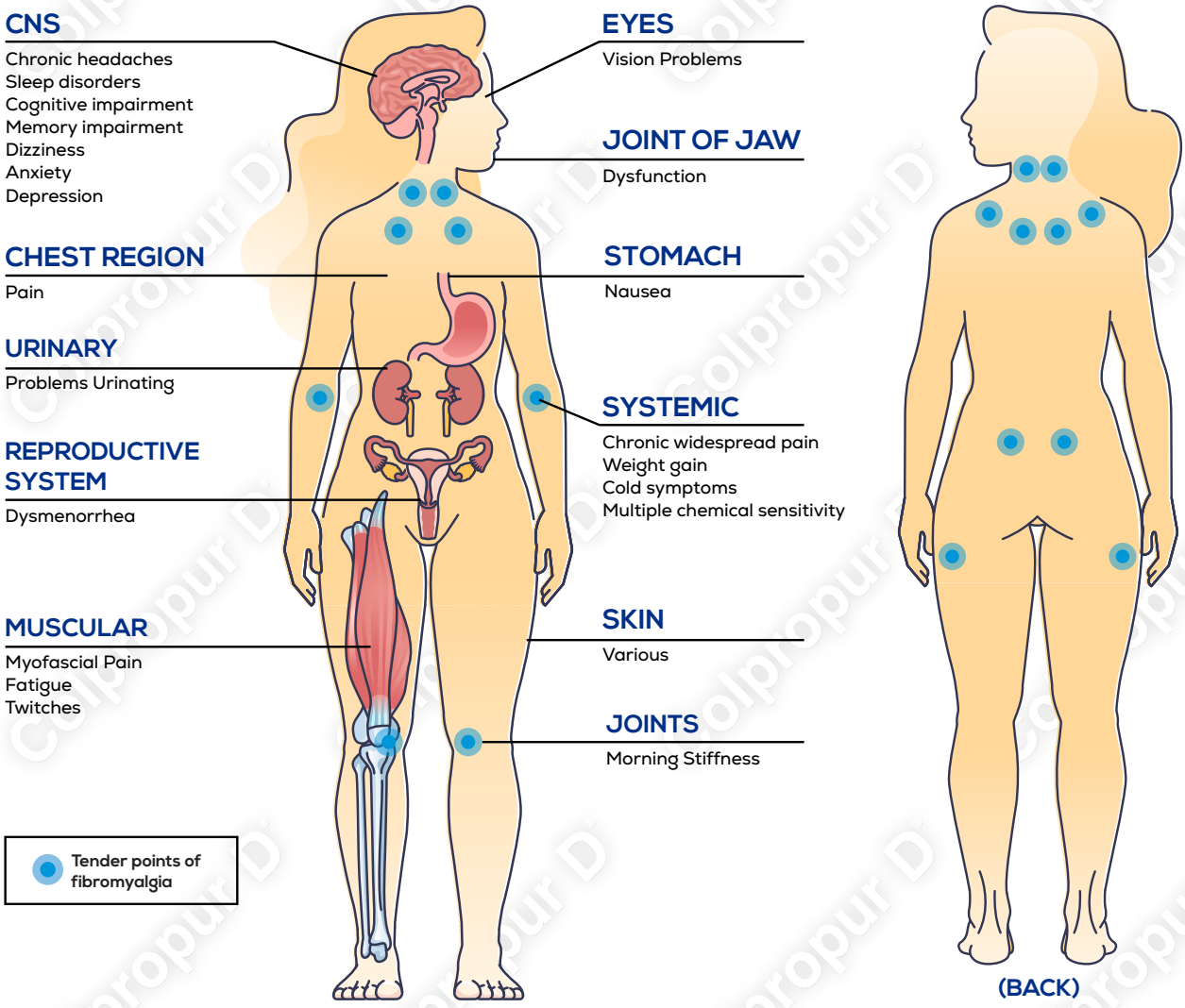
Fig. 20

The authors suggest that FMS symptomatic improvement may be due to the fact that HC not only provides building blocks for cells (collagen-specific AAs), but also helps to balance neuroendocrine functions.

- Other observational study **[89]** is carried out with 20 medium-level FMS patients that take 10g of **COLPROPUR D®** (under Colnatur® brand) daily for three months. The evolution of pain, fatigue, insomnia and the number of pain points are evaluated, using the visual analog scale (VAS) in the first three. The main findings are as follows:

1. 80% of patients show a reduction in pain VAS scores between 30 and 100%, with half of this group between 60 and 100%.
2. 85% of patients show a reduction in the number of pain points between 57 and 83%.
3. 70% of the patients show a reduction in fatigue VAS score between 30 and 100%, and most of this group between 80 and 100%.
4. 45% of the patients show a reduction in insomnia VAS score between 30 and 100%, and most of this group between 75 and 100%.
5. The perception of beneficial effects begins after the first month of **COLPROPUR D®** intake and intensifies later. 65% of participants describe other benefits not quantified in the study based on skin, hair and nails.

In summary, previous studies suggest that HC intake improves some of fibromyalgia symptoms, especially those related to pain, which is consistent with the results of other studies showing that fibromyalgia patients are deficient in collagen concentration and cross-links in intramuscular connective tissue, a condition that produces pain and can be alleviated by stimulating collagen biosynthesis through the continuous daily intake of HC.



Potential disorders and tender painful points characteristic of Fibromyalgia

GLUCOSE METABOLISM AND TYPE II DIABETES MELLITUS (T2DM)

T2DM is an endocrine and metabolic disorder caused by impaired function of insulin leading to chronically high blood sugar levels and serious complications. Current synthetic antidiabetic drugs may cause risk of hypoglycemia, weight gain, pancreatitis and gastrointestinal side effects. Didipeptidyl peptidase-4 (DPP-4) inhibitors are a new therapeutic approach of growing interest. In fact, DPP-4 enzyme degrades incretin hormones, which are released during meals, and stimulate insulin secretion from pancreas β-cells. The use of DPP-4 inhibitors prolongs the half-life of incretins and its hypoglycemic action.

Preclinical studies

In vitro **[90]** and *in vivo* **[91]** studies suggest that the glycemic-lowering effect exhibited by collagen peptides is due to their capacity to inhibit DPP-4.

CLINICAL STUDIES

- Two RCTs **[92,93]** are carried out with 60 T2DM patients of both genders aged between 21 and 50 years who receive a HC supplement or an active control (*Resistant Dextrin*, RD) daily for 3 months. RD is a soluble dietary fiber therapeutically effective since studies have shown that it attenuates postprandial blood glucose level. During the study, participants continue to take their usual oral hypoglycemic agent.

  Results show that HC daily doses of 5 to 10g for 3 months decrease more significantly fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c) **(Fig. 21)** and insulin resistance (HOMA IR) **(Fig. 22)** than RD active control. Intergroup comparison shows that percentage of improvements in FBG, HbA1c and HOMA-IR (% of reduction) is significantly higher after the HC treatment than after the RD treatment **(Fig. 23)**. Both functional food supplements have different mechanism of action: collagen peptides exhibit DPP-4 inhibitory bioactivity and RD viscous fibers delay intestinal glucose absorption acting mechanically.

  Moreover, the combination of oral antidiabetic drug and HC does not result in hypoglycemia in any case and no adverse effect is reported. These clinical studies conclude that HC is a safe and useful option for T2DM management.

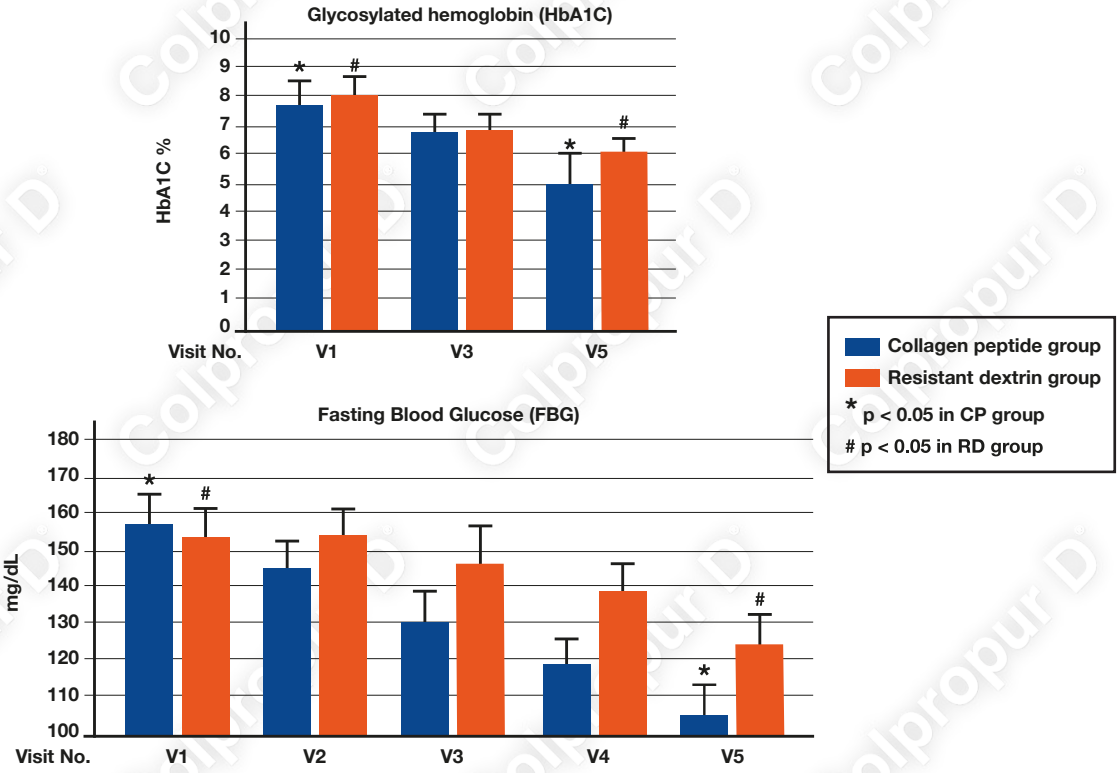


Fig. 21

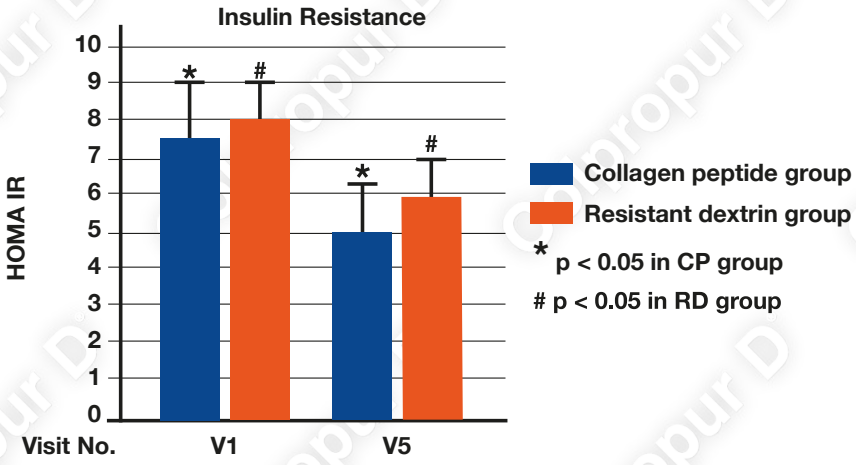


Fig. 22

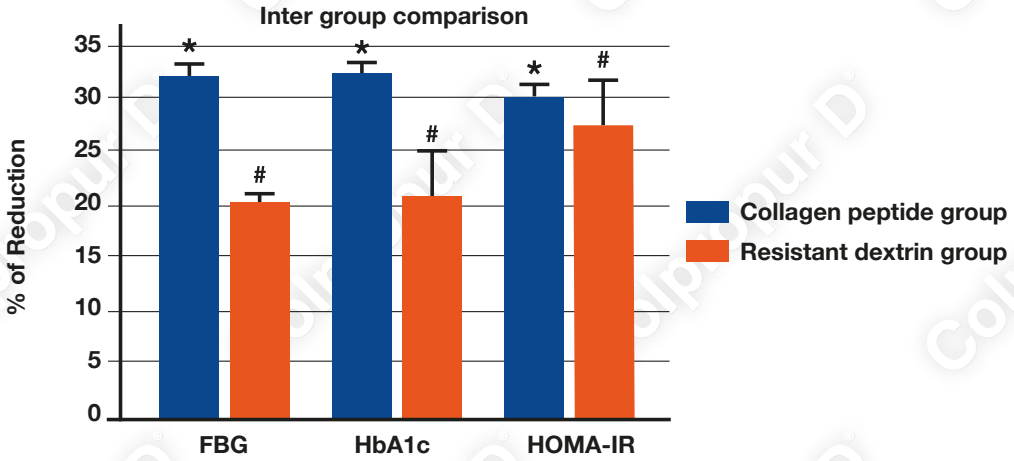


Fig. 23. % of reduction in CP group is significantly higher than in RD group ($p < 0.05$).

- Other RCTs conducted in T2DM patients **[94]** and T2DM patients with primary hypertension **[95]** show that a treatment with 13g of HC daily for 3 months not only improves glucose metabolism, but also lipid metabolism, reducing significantly levels of serum triglycerides, total cholesterol, low-density lipoprotein and free-fatty acids, and increases levels of high-density lipoprotein and adiponectin.

In summary, previous studies show that HC acts as DPP-4 inhibitor, causing a hypoglycemic effect that in no case (e.g. in combination with oral antidiabetic drug) leads to hypoglycemia. The intake of HC is more effective than that of other non-pharmacological components such as resistant dextrin (prebiotic fiber), which acts mechanically by delaying the absorption of glucose while collagen peptides inhibit DPP-4 thanks to their bioactivity.

BLOOD PRESSURE (BP) - LOWERING ACTIVITY

Hypertension ranks as the top cause of cardiovascular disease, the leading contributor to mortality worldwide, and its prevalence has continued to rise in recent decades.

The renin-angiotensin system (RAS) is a hormone system that modulates blood pressure (BP) and balances body fluids and electrolytes. Within RAS, angiotensin-I converting enzyme (ACE) promotes the conversion of angiotensin I to angiotensin II, a powerful vasoconstrictor. ACE inhibitors are widely used as antihypertensive agents. They act by blocking this conversion and, consequently, favoring the dilation of blood vessels and the reduction of BP. However, the prolonged use of synthetic drugs may cause side-effects. Therefore, there is a growing interest in identifying ACE inhibitors from natural sources.

Preclinical studies

In vitro studies show that collagen peptides exhibit ACE-inhibitory activity **[96]**. This explains that in *in vivo* studies HC administration significantly lowers BP in spontaneously hypertensive rats **[97-99]**.

CLINICAL STUDIES

- A RCT **[100]** is carried out with 58 subjects of both genders with a mean age of 53 years and mild hypertension or high-normal blood pressure who have not been treated with any antihypertensive agent. Participants receive a HC supplement (test food group, TFG) or placebo daily for 12 weeks. The results are the following:

  1. After treatment, systolic BP in TFG is significantly lower than at baseline ($p \leq 0.001$) and also compared to placebo group ($p \leq 0.05$). Diastolic BP in TFG shows a decreasing trend comparing to placebo group, which does not experiment any significant change in BP.

  2. Brachial-ankle pulse wave velocity (baPWV) is use as an indicator of arterial stiffness and vascular damage. baPWV values are known to be higher in winter than in summer. After treatment, baPWV average value in placebo group is significantly higher than at baseline ($p \leq 0.01$) (probably because the study takes place between July and December) and than in TFG ($p \leq 0.05$), whose baPWV average value remains without significant changes **(Fig.24)**.

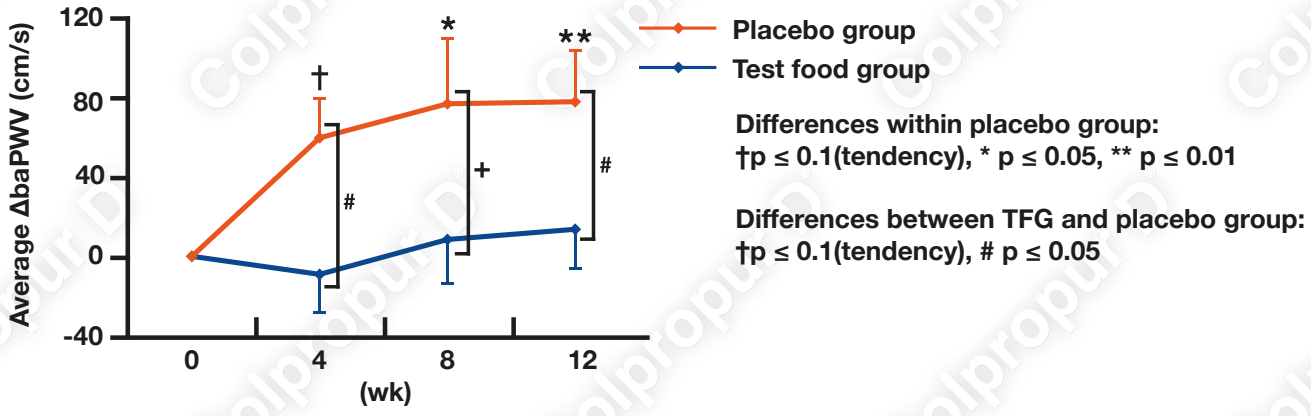


Fig. 24

3. After treatment, serum nitrogen oxide (NOx) value increases more markedly within TFG than withing placebo group. NOx relaxes vascular smooth muscles, expanding arteries and favoring blood flow. It also inhibits platelet adhesion to vascular endothelium **(Fig.25)**.

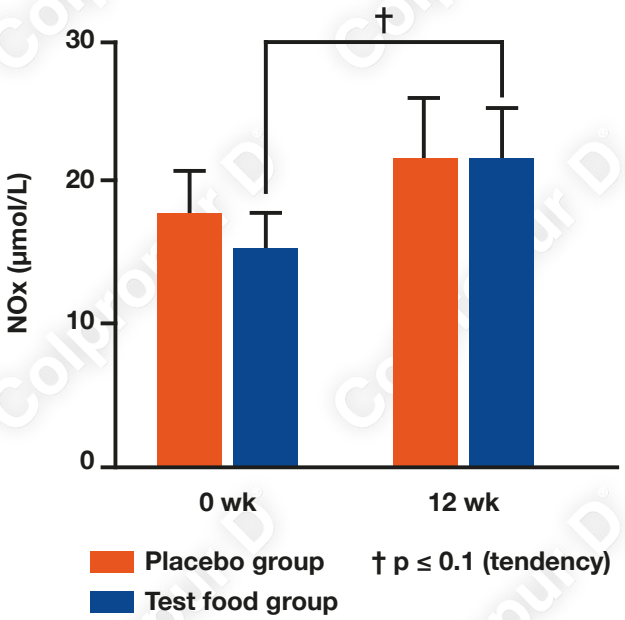


Fig. 25

In summary, previous studies show that HC acts as ACE inhibitor, causing an antihypertensive effect. In addition, studies have observed that HC has a vasoprotective effect, favoring arterial flexibility and vascular relaxation, and preventing platelet aggregation.

ATHEROSCLEROSIS AND CARDIOVASCULAR PROTECTION

Currently, cardiovascular diseases are the leading cause of mortality worldwide. The main risk factors are obesity, diabetes, dyslipidemia and hypertension. Atherosclerosis, widely considered the chief component in cardiovascular pathologies, is characterized by the formation of lipid deposits (plaques) in vascular walls, causing the arteries to progressively harden, narrow and become damaged. Patients usually notice this process only when severe symptoms such as infarction or ischemia occur. Therefore, it is important to identify early risk factors and control them through the evaluation of reliable parameters as baPWV (brachial-ankle pulse wave velocity) and CAVI (cardio-ankle vascular index) to predict arterial stiffness, serum LDL-C/HDL-C (low-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratio) to predict carotid intima-media thickness, and serum TAGE (toxic advanced glycation end-products) to predict vascular inflammation and plaque progression.

Preclinical studies

These studies show the capacity of collagen peptides to inhibit platelet aggregation **[101,102]**, vascular inflammatory response **[103]** and oxidative endothelial dysfunction **[104]**.

CLINICAL STUDIES and META-ANALYSIS

Some clinical studies **[105,106]** have observed that collagen peptides can improve several predictors of atherosclerosis, helping to prevent the onset and worsening of this disease. Furthermore, a meta-analysis **[107]** reveals that HC intake helps to reduce several risk factors for cardiovascular disease.

- A RCT **[105]** is carried out with 64 healthy subjects of both genders with a mean age of 73 years. Participants receive HC supplement or placebo daily for 12 weeks. After the treatment, baPWV values decrease significantly within treatment group from baseline and also compared to placebo group ($p < 0.01$ in both cases), but they remain without significant changes within placebo group. This result suggests that collagen peptides could be useful for improving arterial flexibility and, thus, preventing the progression of atherosclerosis.

- An observational study **[106]** is carried out with 30 healthy subjects of both genders with a mean age of 54 years. Participants receive HC supplement daily for 6 months. After the treatment, the main findings are as follows:

    1. LDL-C/HDL-C ratio **(Fig. 26)** and TAGE levels **(Fig. 27)** significantly decrease within the high risk group from baseline to 6 months ($p < 0.05$ both cases), suggesting that HC can be especially helpful when there is a propensity for plaque and vascular damage.

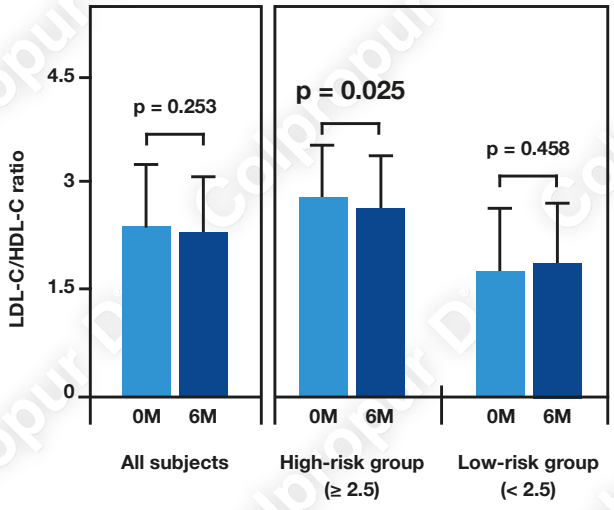


Fig. 26

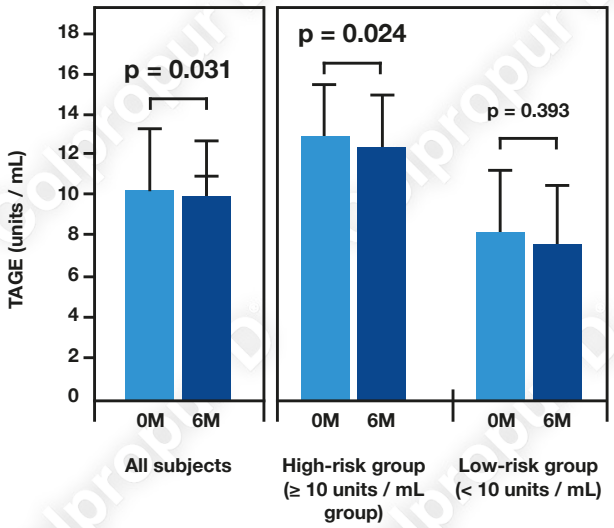


Fig. 27

2. CAVI values significantly decrease in all the subjects from baseline to 6 months ($p < 0.05$), indicating a recovery of blood vessel elasticity following HC treatment. In fact, CAVI increases by approximately 0.5 points for every additional 10 years of age. Therefore, the 0.2-point decrease observed in this study suggests that 6 months of HC daily intake exerts a significant preventive effect, leading to a recovery of blood vessel elasticity equivalent to 4 years less.

- A systematic review and meta-analysis of 12 selected RCTs **[107]** shows that collagen peptide supplementation significantly reduce fat mass, low-density lipoprotein and systolic blood pressure, while increasing fat-free mass. These effects lead to a decrease in cardiovascular risk.

In summary, previous studies show that HC acts directly on the vascular wall, exerting a vasoprotective activity against atherosclerosis. This, along with its antihypertensive, lipid-lowering and glycemic-lowering effects, can improve cardiovascular health and become an important component of preventive medicine.

GASTROINTESTINAL PROTECTION

Preclinical studies

a) Anti-ulcerogenic activity

Invasive factors including smoking, drinking, helicobacter pylori infection, NSAIDs consumption and psychological stress can damage the gastric mucosa (GM), leading to ulcers in severe cases.

- An *in vivo* study **[108]** performed with a stress-induced ulcer rat model shows that collagen peptide administration (Pro-Gly-Pro, PGP, and N-acetyl-Pro-Gly-Pro, AcPGP) can reduce the total area of GM damage by 63% (Fig.28), and prevent the increase in stress hormones and proinflammatory cytokines levels. Results suggest that HC is useful for moderating the intensity of neuroendocrine and immune responses to stress and the consequential GM damage.

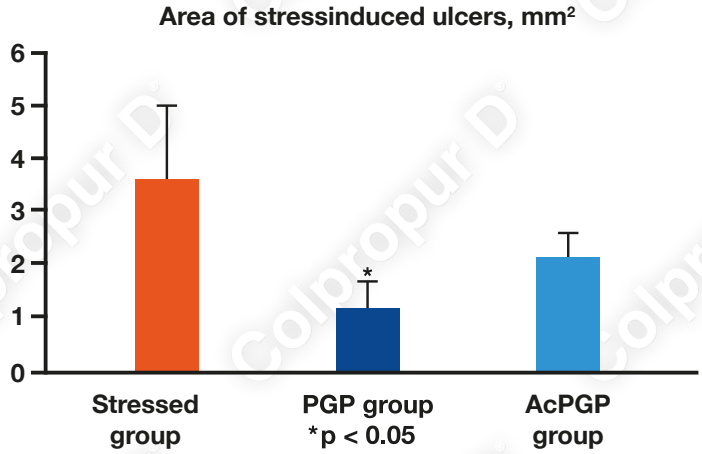
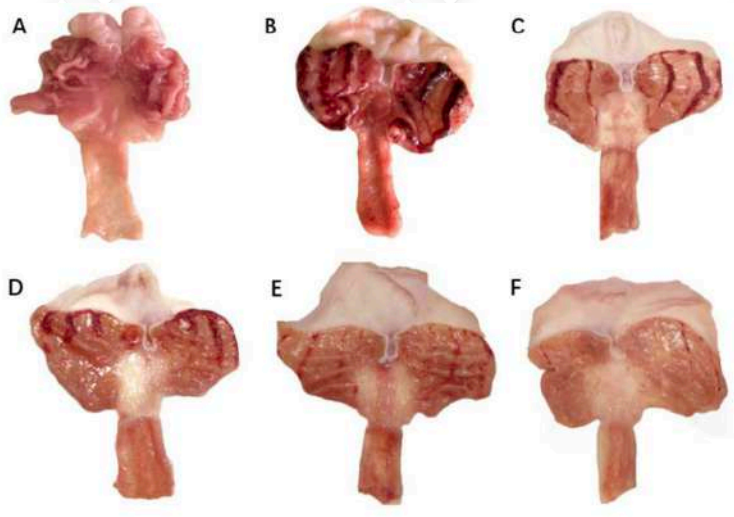


Fig. 28

- Other *in vivo* study **[109]** performed in ethanol-induced ulcer rat model shows that collagen peptide administration significantly reduces gastric and duodenal ulcer index **(Fig. 29)** and increases gastric juice pH, along with the improvement of others biomarkers in gastric and duodenal tissues, and in serum. Results suggest that HC can protect mucous membrane barrier and improve microcirculation through antioxidant, anti-inflammatory and anti-apoptotic effects.



A: normal control group
B: ethanol group
C: whey protein control group (250 mg/kg BW)
D: HC low dose group (250 mg/kg BW)
E: HC medium dose group (500 mg/kg BW)
F: HC high dose group (1000 mg/kg BW)

Fig. 29. Degree of gross gastroduodenal injury.

- Finally, a 2-period, 2-treatment crossover study **[110]** is carried out with 10 stall-confined horses which receive daily 90g of porcine HC in two doses for 56 days. Results show that HC feeding enhances the effects of omeprazole, increasing gastric juice pH significantly. Also, it inhibits serum gastrin concentrations (systemic effect) and leads to fewer nonglandular ulcers (local mucosal effect). The HC mixed with grain is readily consumed by all horses and no adverse responses are observed.

b) Gut microbiota modulation

It is well known that gut microbiota is involved in immune response and intestinal epithelial barrier functions. Now, emerging evidence connects gut microbiota with OA, obesity and metabolic disorders.

- An *in vitro* study **[111]** suggest that enzymatic hydrolysis of HC in the stomach and small intestine yields some peptides that escape intestinal absorption and undergo colonic fermentation, inducing prebiotic effects that modulate gut microbiota composition.

- *In vivo* studies show that collagen peptides improve gut microbiota in immunosuppressed and high-fat diet (HFD) mice models **[112,113]** by increasing short-chain fatty acids concentration (an indicator of a healthy microbial community) and the relative abundance of beneficial bacteria versus pathogenic and inflammation bacteria.

- In a HFD mice model **[113]**, an 8-weeks treatment with a dose of 800 mg/kg of collagen peptide (CP) achieves an effect similar to a treatment with a dose of 40 mg/kg of Orlistat (ORL), the only drug recognized for weight loss worldwide. Under a HFD, both CP and ORL treatments lead to a reduction in body weight accumulation **(Fig. 30)** by similarly improving lipid metabolism and gut microbiota composition.

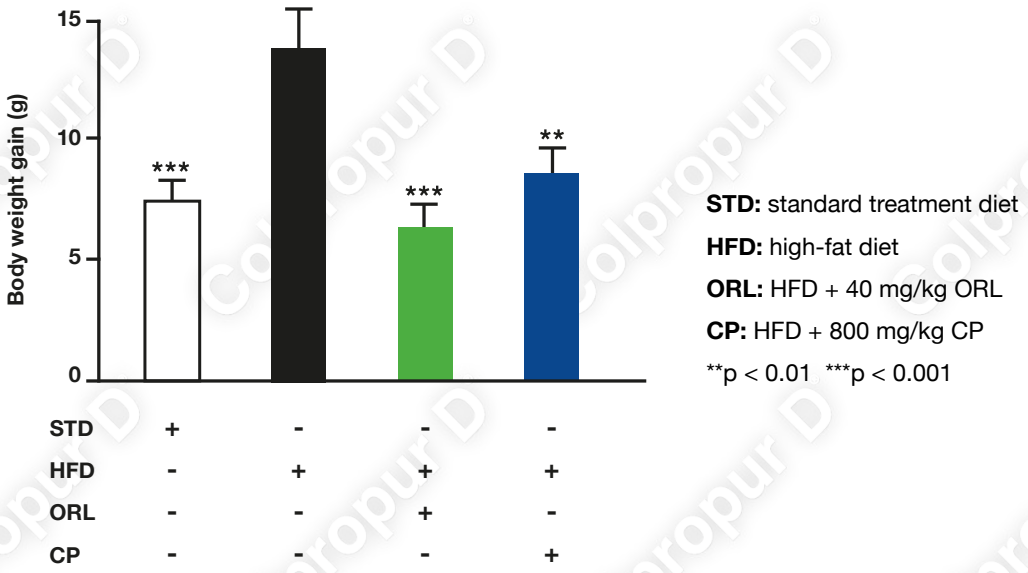


Fig. 30. Changes in body weight accumulation.

CLINICAL STUDIES

It is estimated that > 40% of the global population experiences at least one digestive disorder that include bloating, stomach cramps, pain, diarrhea, constipation, flatulence, irregular bowel movements and acid reflux.

- An observational 2-phase mixed methods study **[114]** is carried out with 14 healthy adult women with a mean age of 46 years and overweigh. Participants suffer from digestive symptoms such as bloating, irregular bowel habits, flatulence, stomach cramps and acid reflux.

Phase 1 consists of a 2-week pre-intervention period with monitoring of diet, lifestyle and digestive symptoms, and completion of the Medical Symptoms Questionnaire (MSQ 1).

Phase 2 consists of 8-weeks daily supplementation period with 20g of HC (bovine collagen peptides) in two doses. Participants complete MSQ 2 after the first 2 weeks of supplementation, and MSQ 3 after the total of 8 weeks of supplementation.

The results of MSQ scores **(Fig. 31)** show a general improvement in digestive symptoms (-8%), with a reduction mainly in intestinal stomach pain (-39%) **(Fig. 32)** and bloating (-31%) **(Fig. 33)**, followed by a reduction in acid reflux (-21%) and constipation (-19%).

	Baseline score, mean (SD; 95% CI)	Week 2 score, mean (SD; 95% CI)	Week 8 score, mean (SD; 95% CI)	Change, baseline to week 8 (%)
Total MSQ score	61.2 (11.1; 54.4-67.9)	64.6 (4.7; 61.8-67.5)	56.4 (13.5; 48.3-64.5)	-8
Bloating	3.9 (0.4; 3.7-4.1)	3.2 (1.0; 2.7-3.8)	2.6 (1.1; 2.0-3.3)	-31
Constipation	2.2 (1.0; 1.7-2.8)	2.2 (0.8; 1.8-2.7)	1.8 (1.0; 1.2-2.4)	-19
Intestinal stomach pain	2.6 (0.7; 2.2-3.0)	1.9 (0.5; 1.7-2.2)	1.6 (0.8; 1.1-2.0)	-39
Acid reflux	2.1 (1.0; 1.5-2.7)	2.0 (1.0; 1.5-2.6)	1.6 (1.2; 1.0-2.3)	-21

Fig. 31. Absolute and percentage change in Medical Symptoms Questionnaire (MSQ) scores.

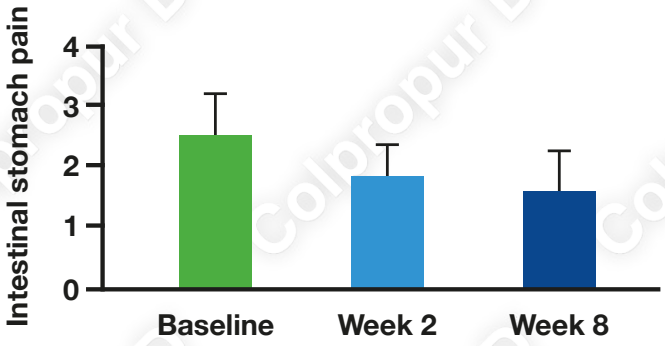


Fig. 32

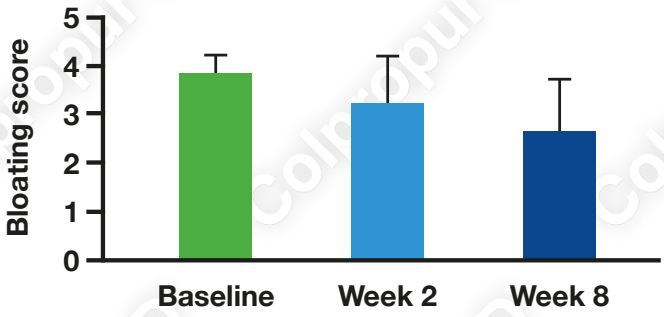


Fig. 33

Average scores in Fig.32 and Fig.33 are calculated within MSQ 5-point scale of symptom occurrence and its effect: 0=never or almost never; 1=occasional but not severe; 2=occasional, severe; 3=frequent, not severe; and 4=frequent, severe. Data are expressed as mean (SD).

In summary, previous studies show that HC intake protects gastric mucosa, exhibiting anti-ulcerogenic activity and improving digestive disorders. In addition, HC intake protects intestinal epithelium functions and exhibits gut microbiota beneficial effects, improving the metabolic and immune functions in which it is involved.

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Currently there are more than 400 scientific publications showing HC health and technical properties.

This is a selected bibliography extracted from the extended and continued updated collagen bibliography you can find in our website www.colpropur.com.

Studies 5-7, 14, 27 (PHOSCOLLAGEN®), 53, 65, 86 and 89 have been carried out with COLPROPUR D® HC (under different brands or presentation forms).

COLPROPUR D® HYDROLYZED COLLAGEN

COLPROPUR D® is a totally soluble HC in powder form, a pure ingredient with maximum food safety and tolerance. Collagen comes from traceable raw materials that constantly respect the cold chain and food standards. It is obtained by an exclusive process, in which only physical and enzymatic means are used (without the addition of chemical products that could cause the presence of residues in the final product), and whose safety has been specifically approved by the European Food Safety Authority (EFSA Q 2004-085).

COLPROPUR D® provides collagen specific amino acids and bioactive peptides in an immediate and very bioavailable way, acting mainly at two levels: **1.** Stimulating the endogenous collagen biosynthesis and nourishing connective tissues helping to reduce, prevent and slow down their deterioration. **2.** Acting as biological signals (biopeptides) modulating several physiological functions whose compliance is beneficial to relevant health aspects as immunology, metabolism, cardiovascular and gastrointestinal functions, and gut microbiota. **COLPROPUR D®** has the following properties:

EFFICIENT
Perceptible benefits from 1 to 3 months

0% WITHOUT
fats, sugars, sweeteners, colorants, preservatives or GMOs

SAFE
Does not require a break.
Pregnant women and children can take it

SCIENTIFICALLY PROVEN BENEFITS

PURE
Without chemical residues

ASSIMILABLE
(Hydrolyzed)

NO INTOLERANCES OR ADVERSE EFFECTS

NATURAL
in origin, method of obtaining and mode of action

COMPATIBLE
with medications and dietary supplements

Taking 10 grams/day of COLPROPUR D® is a recommended health habit for all ages and in all circumstances, particularly in groups with special health needs (over 40, athletes, musculoskeletal diseases, etc.), due to: **1.** The absence of assimilable collagen in the current diet. **2.** The need for long-lasting harmless alternatives to help to treat chronic and degenerative diseases of the musculoskeletal system, such as osteoarthritis, osteoporosis, ligament and musculotendinous injuries, fibromyalgia and sarcopenia.

From a nutritional point of view, **COLPROPUR D®** provides 60% of conditionally essential amino acids (CEAAs), as glycine, proline and arginina. Although they can be synthesized from EAAs, CEAAs become essential under conditions of stress (intense physical activity, cellular aging and catabolic states: critical illness, trauma, infection, gastrointestinal disorders...), when the capacity of endogenous amino acid synthesis is exceeded and this fact can lead to the onset or aggravation of a growing number of varied diseases. **COLPROPUR D®** is an excellent source of CEAAs and bioactive peptides that helps to avoid this deficit and its long-term consequences.

In short, **COLPROPUR D® exerts a beneficial systemic effect** on immune, metabolic, cardiovascular and gastrointestinal health, **and a beneficial local effect** (on connective tissue health), **being an essential food ingredient to obtain a balanced protein intake between EAAs, CEAAs and non-EAAs.**

COLPROPUR D® aminogram

Grams of amino acids per 100 g of protein								Amino acid types	
Glycine[C]	22.4	Arginine[C]	7.5	Valine*	2.4	Methionine*	0.8	EAAs (*)	16%
Proline[C]	13.2	Aspartic acid	5.5	Phenylalanine*	2.3	Histidine*	0.5		
Hydroxyproline[C]	11.9	Lysine*	4.0	Thréonine*	1.7	Tyrosine[C]	0.4	CEAAs (C)	60%
Glutamic acid	10.1	Serine[C]	3.2	Isoleucine*	1.3	Triptophan*	0.1		
Alanine	8.8	Leucine*	2.8	Hydroxylysine[C]	1.1	Total	100	Non-EAAs	24%

DIAGRAM OF ELABORATION, ASSIMILATION AND BIOACTIVITY

NATIVE COLLAGEN in animal tissues
300.000 Da

↓

Extraction and gelatinization or denaturation of native collagen

↓

GELATINE or DENATURED COLLAGEN
(random mix of coiled chains)
100.000 Da

↓

Exogenous enzymatic hydrolysis

↓

HYDROLYZED COLLAGEN
(small peptides pool)
< 5.000 Da

↓

Oral intake

↓

Endogenous digestive hydrolysis

↓

Intestinal absorption

↓

COLLAGEN AAs, DI and TRIPEPTIDES

connective tissues

Basic bioactivity

Biological signals and building blocks for ECM formation

Stimulation and biosynthesis of connective tissues regeneration of:

- BONES
- JOINTS
- TENDONS and LIGAMENTS
- MUSCLE FASCIA
- SKIN
- BLOOD VESSELS

Special bioactivities

Biological signals for physiological processes

- Immunomodulatory, antiinflammatory and antioxidant activity
- Body composition and physical activity performance improvement
- Gastrointestinal protection and gut microbiota modulation
- Skin wound healing improvement
- Lipid-loweing activity
- Anti-atherosclerotic activity (platelet aggregation inhibitor)
- Anti-hypertensive activity (ACE-inhibitor)
- Anti-diabetic activity (DDP-IV inhibitor)
- Neuroprotective and antidepressant activity

MEDICAL SPECIALTIES INVOLVED

GINECOLOGY

The drop in estrogen level due to menopause causes a marked slowdown in endogenous collagen biosynthesis. Consequently, an accelerated deterioration of the connective tissues takes place during postmenopause. **COLPROPUR D®** intake can help to counteract this process, contributing to prevent and reduce joint pain, bone mass loss and skin aging.

GERIATRICS

The progressive decrease in endogenous collagen production associated with aging is the cause of the worsening of musculoskeletal degenerative diseases in advanced ages. **COLPROPUR D®** intake can help in the prevention and management of osteoarthritis, osteoporosis, sarcopenia and pressure ulcers, contributing to avoid their aggravation and making their evolution less drastic.

SPORTS MEDICINE

COLPROPUR D® intake can help to maintain the good condition of the conjunctive tissues involved in sports practice and facilitates their recovery after physical effort. Specifically, it can help to prevent accelerated joint wear and ligament, tendon and muscle chronic injuries. In addition, it can contribute to improve sport performance and body composition, increasing fat-free mass and strength.

DERMATOLOGY

COLPROPUR D® intake can help to improve skin properties and functions, preventing and reducing skin aging and photoaging signs, and protecting epidermal barrier function from UVB radiation damage. Furthermore, it can contribute to accelerate the healing of wound of different origins: burns, surgical incisions and pressure ulcers.

TRAUMA AND PHYSICAL REHABILITATION

Thanks to its beneficial effects on conjunctive tissues, **COLPROPUR D®** intake can help to consolidate the result of trauma surgery, prosthesis, implants, etc. and also contribute to a better recovery. It is highly recommended to start the intake as long as possible before the intervention (3 months would be ideal). In addition, it can help to enhance the results of physical rehabilitation techniques.

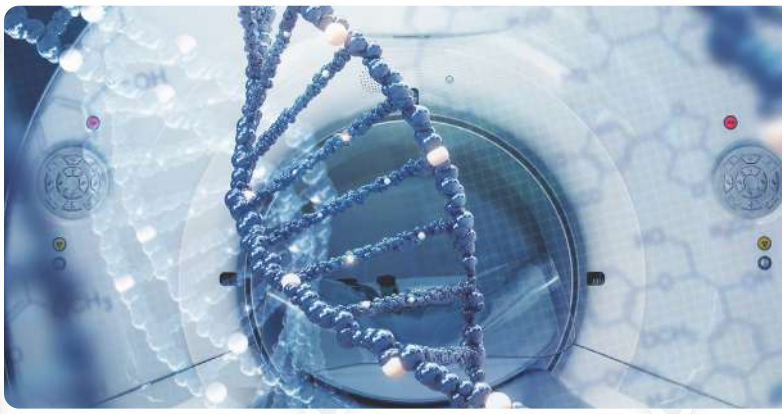
RHEUMATOLOGY

COLPROPUR D® intake can help to prevent and manage chronic degenerative diseases as osteoarthritis (OA) and osteoporosis (OP). In OA, it can contribute to alleviate join pain, allowing rescue medication to be reduce. In OP, it can stimulate bone formation, complementing the action of antiresorptive drugs. Of special interest is **PHOSCOLLAGEN®**, a HC-hydroxyapatite complex that has shown its osteogenic activity in vitro **[27]**. In addition, it has been shown that hydrolyzed collagen has antiinflammatory activity and increases oral tolerance, helping to improve autoimmune diseases (arthritis and ulcerative colitis).

ODONTOLOGY AND ORAL SURGERY

COLPROPUR D® intake can help to strengthen conjunctive tissues of oral cavity, as gums and jaw bone, helping to consolidate dental implants and improve the result of dental surgery. It is highly recommended to start the intake as long as possible before the intervention (3 months would be ideal).

REGENERATIVE MEDICINE



Thanks to its nutritional contribution (collagen-specific AAs, especially glycine, proline, and hydroxyproline) and to the bioactivity of small collagen peptides, **COLPROPUR D®** intake can promote the regeneration of connective tissues, stimulating formation versus resorption in their turnover balance. This action can enhance the results of regenerative techniques, such as rich plasma in growth factors, stem cells and biomaterial implants.

IMMUNOLOGY AND ONCOLOGY

Skin and connective tissues have an important immunological function: they form physical barriers that hinder the entry, circulation and expansion of infectious agents and tumor cells inside the body. **COLPROPUR D®** intake can help to strengthen connective tissues, enhancing its immunological function and minimizing cancer treatment tissue damage. In addition, it has been shown that collagen peptides have immunomodulatory activity. Specifically, in an in vitro study **[65]**, **COLPROPUR D®** hydrolyzed collagen has shown to stimulate immune cells proliferation and modulate inflammatory response to be effective and not exacerbated.

Colpropur D®

Protein

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