An Overview of the Beneficial Effects of Hydrolysed Collagen as a Nutraceutical on Skin Properties: Scientific Background and Clinical Studies

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An Overview of the Beneficial Effects of Hydrolysed Collagen as a Nutraceutical on Skin Properties: Scientific Background and Clinical Studies

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Abstract: Skin, the main barrier to the external environment, is subject to deterioration caused by dermatological disorders, environmental conditions and the intrinsic ageing process. This damage to both structure and function may be accelerated by smoking, alcohol consumption and chronic sun exposure (extrinsic components). All these factors may lead to the formation of wrinkles, the appearance of brown spots and skin thickening. One effective strategy to managing the skin ageing process is adopting a healthy nutritional approach to life, maintaining a balanced diet and a good supply of food supplements. This can restore the homeostasis of macro and micronutrients and support the physiology of cells and tissues in the skin. Hydrolysed collagen, an increasingly popular nutraceutical, is composed of low molecular weight small peptides, which are easily digestible, absorbed and distributed in the human body. Numerous clinical trials have now been performed showing the efficacy and benefits of collagen peptides on skin properties, such as hydration, elasticity and reduction of wrinkles. As a result, hydrolysed collagen can be considered an important weapon in the everyday fight against skin ageing.

Keywords: Anti-ageing, hyaluronic acid, hydrolysed collagen, nutraceutical, nutritional supplement, skin, wrinkles.

INTRODUCTION

This review article examines the available data with regard to the use of hydrolysed collagen as a nutraceutical in skin ageing.

Below we bring together a thorough review of the basic physiology of collagen formation and its degradation during skin ageing and the beneficial effects of oral ingestion of hydrolysed collagen on skin properties such as hydration, elasticity and reduction of wrinkles.

COLLAGEN

Collagen is the main structural protein of the different connective tissues present in animals. It is mostly found in fibrous tissues, such as tendons and ligaments, and is also abundant in the cornea, cartilage, bones, blood vessels, the gut, and intervertebral discs (Table **1**). Collagen is the major insoluble fibrous protein found in the extracellular matrix of the skin, together with elastin and hyaluronic acid. The collagen family consists of 28 different proteins [1, 2], which account for 25% - 35% of the total protein mass in mammals and play a pivotal role in the structure of several tissues, such as skin and bones, providing rigidity and integrity [3, 4]. The extracellular matrix (ECM) of connective tissues is formed by diverse protein families involved in the structural integrity and several physiological functions. Composition and structure of the ECM vary considerably in the different types of connective tissues and result in unique functional and biological characteristics [5].

Based on their structure and three-dimensional organization, the collagen family of proteins can be grouped into fibril-forming collagens, fibril-associated collagens (FACIT), network-forming collagens, anchoring fibrils, transmembrane collagens (MACIT), basement membrane collagens and others with unique functions [2, 5].

In the human body 80 - 90% of the total collagen consists of the fibril-forming collagens. Type II and XI collagen fibrils are involved in the formation of the fibrillar matrix in the articular cartilage [5-7].

Type IV collagen, in contrast, forms a two-dimensional reticulum and this more flexible triple helix is restricted to basement membranes [5, 8].

Multiple triple helices form a collagen fibril and multiple fibrils pack together to form a collagen fibre (Fig. (**1**)). Generally collagen fibrils are made of different collagen types: collagen I and III in the skin; collagen II and III in cartilage [4]. The diversity of the collagen family (collagen I, II, III) is mainly determined by the existence of several α-chains with different number of amino acids. Type I collagen

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Table 1. The five most common types of collagen.

Type	Location
Type I	dermis, tendon, ligaments and bone
Type II	cartilage, vitreous body, nucleus pulposus
Type III	skin, vessel wall, reticular fibres of most tissues (lungs, liver, spleen …)
Type IV	forms the basal lamina, the epithelium-secreted layer of the basement membranes
Type V	lung, cornea, hair, fetal membranes and bones

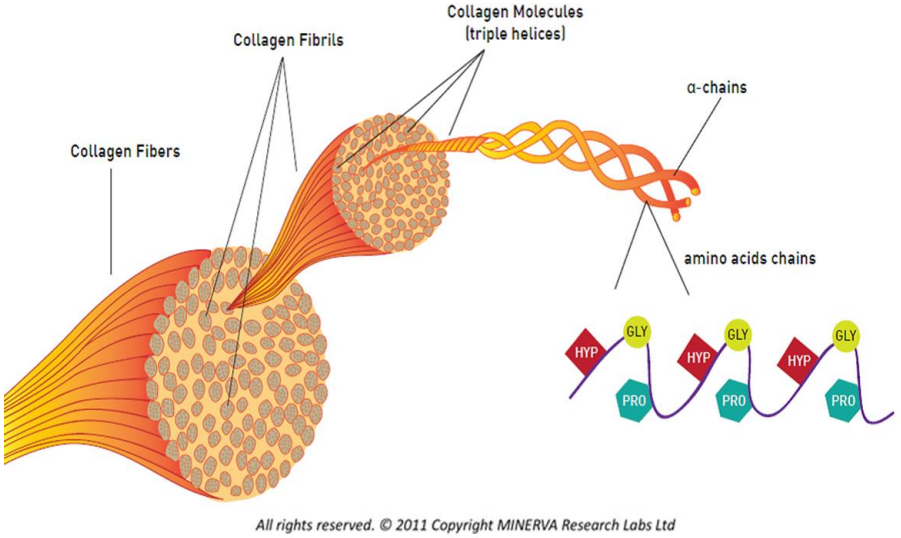

Fig. (1). Structure of the collagen fibres.

is the most abundant in the human body: it forms more than 90% of bone organic mass and it is the major collagen of tendons, ligaments, cornea and many interstitial connective tissues. It is also the main component of human skin (80%) with collagen type III making up the remainder of skin collagen (15%) [5, 9].

The unique physical properties of collagen fibres confer structural integrity to the skin forming a dense network throughout the dermis. The main function of this network is to provide structural support to the epidermis. In addition, collagen and elastin together form the extracellular matrix, which gives the skin its structure, elasticity and firmness [10] (Fig. (**3**)).

Collagen is mainly produced by fibroblasts in the connective tissues [2] but also numerous epithelial cells make certain types of collagens. The different collagens and the structures they form have the purpose to help tissues resist stretching. Fibroblasts are connective tissue cells in the dermis which are responsible for producing and organising the collagen matrix. Fibroblasts are sensitive to physical and chemical stimuli, which can induce both fibroblast activation and proliferation. Chemical stimuli are based on a "key-lock" mechanism where small ligands bind receptors located on the fibroblast extracellular membrane inducing their activation [11]. Physical stimuli are directly related to the interactions between collagen and fibroblasts. The activation of fibroblasts results in an increase in the production of collagen (Fig. (**2**)).

SKIN AGEING

Skin is subject to deterioration caused by dermatological disorders, environmental conditions (wind, air conditioning, heating) and the intrinsic ageing process, which may be accelerated by exposure to sun (photo-ageing [12, 13]) or by other lifestyle issues (extrinsic ageing: smoking, alcohol, stress, lack of sleep and chronic sun exposure [14, 15]). All these factors may cause the formation of wrinkles, the appearance of brown spots and the thickening of the skin [12, 13]. Interestingly, alteration in diet can change the way skin functions as evidenced by the effects of dietary deprivation on skin health. For example essential fatty acid deficiency [16] or accumulation of abnormal fatty acids [17] results in so-called skin scaling and poor barrier function. Moreover, a recent publication shows that in a placebo controlled double blind study the addition, in the diet, of the omega-3 oils from flaxseed and omega-6-rich oils from the borage plant leads to a decrease in skin roughness and scaling, ($P < 0.05$) [18].

Autoimmune diseases, ageing and stress can change the quantity and integrity of collagen in the skin as they impair collagen quality and consequently affect the overall skin function. Studies have also shown that collagen synthesis varies at the different stages of life. Moreover, the relative

proportion of the collagen types changes in skin with age. Young skin is made by 80% type I collagen and about 15% collagen type III. With age, the ability to replenish collagen naturally decreases by about 1.5% per year. Collagen fibres, in aged skin, become thicker and much shorter, resulting in a loss of type I collagen, which alters the ratio of collagen types [19]. The density of collagen and elastin in the dermis declines, hence the structure and elasticity of the skin degrades, causing it to become thinner and more rigid. The ageing process results also in the loss of hyaluronic acid. This reduces the moisture, suppleness and elasticity of the skin. The diminished elasticity of the skin reduces its ability to retain its shape and it does not conform as closely to the contours of the face. The skin appears looser and sags. Lines and furrows emerge to enable movement. Gravity then pulls on the skin, all leading to sagging eyelids, bags under the eyes and jowls.

Skin ageing is a complex biological process which affects several constituents of the skin and hence its appearance. The two primary processes of skin ageing, intrinsic and extrinsic, are controlled, respectively, by genetic variations and by extrinsic components (Fig. (**4**)) [20]. Skin ageing alterations are due to structural changes in the skin cells and in the texture of the dermal tissue, because of the action of free radicals (reactive oxygen species, ROS) produced mostly by UV radiation and to a certain extent by cellular metabolism. ROS induce a molecular destruction and consequently the loss of biological functions [21].

Another important phenomenon that takes place, especially in tissues very rich in proteins, is the production of AGEs (*Advanced Glycation End products*). These molecules

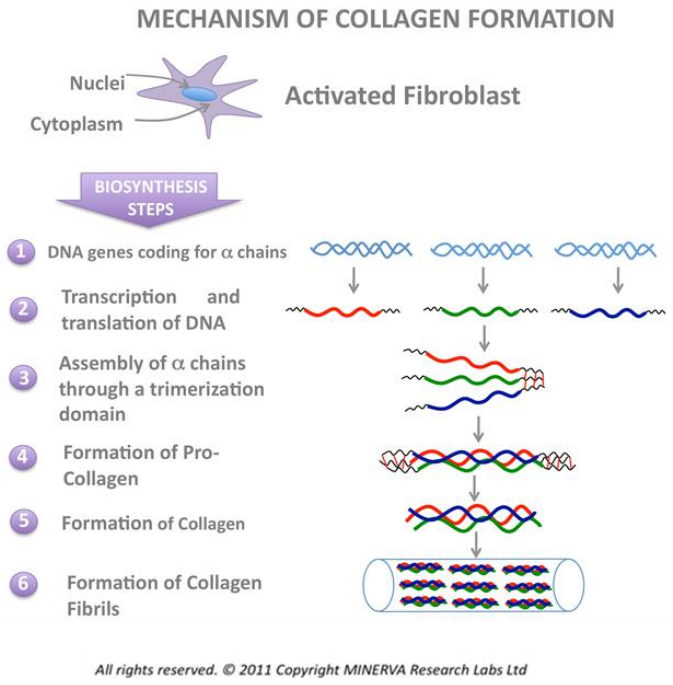

Fig. (2). Collagen synthesis.

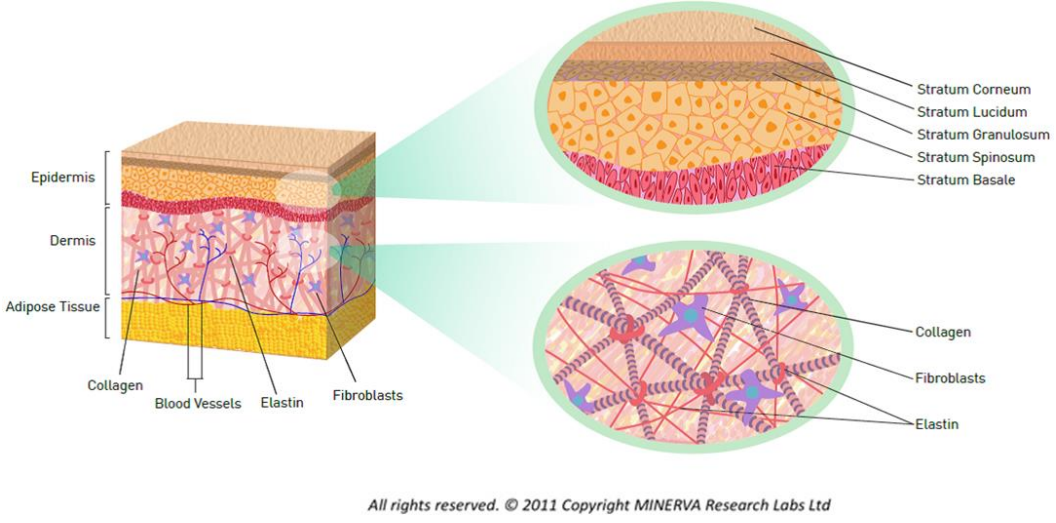

Fig. (3). Diagram showing the structure of healthy skin, in which the different layers are visible: epidermis, dermis and adipose tissue. Collagen fibres, elastin and fibroblasts are also represented in the diagram.

form because of a chemical reaction between glucose and the free amino groups in proteins and they remain in the tissue where they form for long periods because they cannot be degraded normally by enzymes [22]. Moreover, compared to young adult fibroblasts, aged fibroblasts synthesize lower levels of collagen, both *in vitro* and *in vivo* [23]. An ageing dermis is characterized by changes in the production and formation of collagen and elastin fibres. In fact, photo-aged dermis contains disorganized collagen fibres and accumulated abnormal elastin [24, 25]. Also smoking leads to changes in the components of the dermis. Therefore smoking can cause premature ageing of the skin [22, 26]. In addition, several studies have correlated the release of stress hormones, such as glucocorticoids (GC), to changes in the synthesis and degradation of collagen in the skin. An important indicator of the deleterious effects of chronic stress is the dysregulation of the circadian cortisol/ corticosterone rhythm [27, 28]. In response to stress, corticotrophin-releasing factor initiates a cascade of events that end up in the release of GC from the adrenal cortex. GC are able to affect the quality of skin through modulation of the immune system [29]. In the short term, GC release may play a key role in the survival of an organism but its excess can lead to negative effects on almost all tissues [30] and accelerate the ageing process [31].

One answer to the ageing process affecting skin condition and function because of lifestyle, dieting or time passing by, is having a correct nutritional approach, maintaining a balanced diet and a good supply of food supplements to restore the homeostasis of macro- and micronutrients to support the physiology of cells and tissues in the human body.

HYDROLYSED COLLAGEN

Hydrolysed collagen consists of small peptides with low molecular weight (0.3 - 8 kDa), produced from native collagen which is found in bones, skin and connective tissue

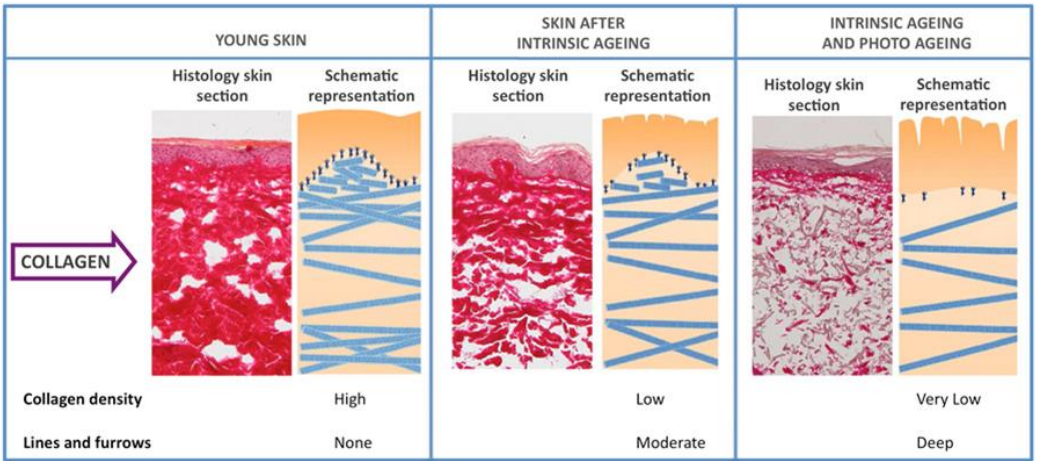

Fig. (4). Collagen decreases after intrinsic and extrinsic ageing [14].

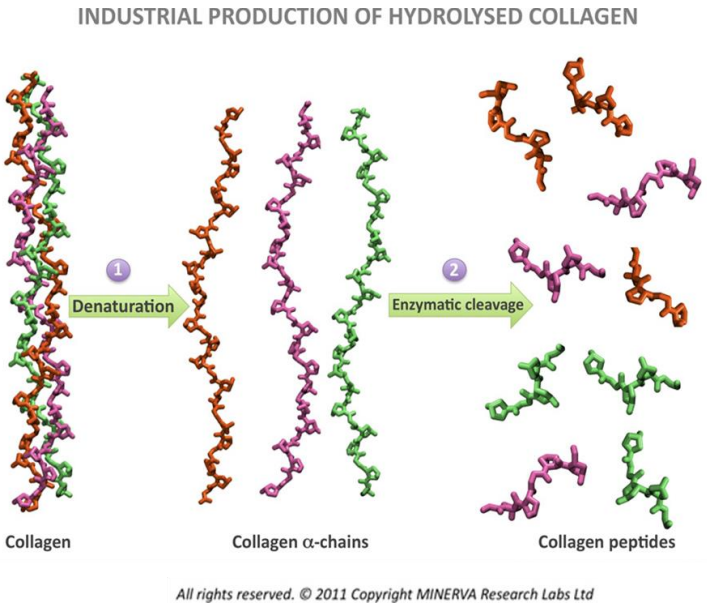

Fig. (5). Industrial production of hydrolysed collagen.

of animals (i.e. cattle, pigs and fish). Due to its low molecular weight, hydrolysed collagen is easily digestible, absorbed and distributed in the human body.

The quality of the final hydrolysed collagen is dependent on its average molecular size, which can vary based on the methodology used to extract it. Generally, collagen molecules are denatured and partially hydrolysed to form gelatin (100 kDa). Gelatin can then be decomposed into small peptides using specific enzymes with cleavage activity (proteinase) (Fig. (**5**)). The molecular weight distribution of collagen peptides usually span in the range 0.3 - 8 kDa. Due to the low molecular weight, there are several advantages of using hydrolysed collagen with respect to native collagen:

- hydrolysed collagen is highly digestible;
- hydrolysed collagen is easily absorbed and distributed in the human body.

When administered orally, hydrolysed collagen reaches the small intestine where it is absorbed into the blood stream, both in the form of small collagen peptides and free amino acids. Through the network of blood vessels, these collagen peptides and free amino acids are then distributed in the human body, in particular to the dermis, where it has been proven they can remain up to 14 days [32]. In the dermis, hydrolysed collagen has a dual action mechanism: 1) free amino acids provide building blocks for the formation of collagen and elastin fibres; 2) collagen oligopetides act as ligands, binding to receptors present on the fibroblasts' membrane and stimulate the production of new collagen, elastin and hyaluronic acid.

Hydrolysed collagen is enriched in specific amino acids: glycine, proline and hydroxyproline. Each amino acid has a particular function. The amino acid profile is shown in (Fig. (**6**)).

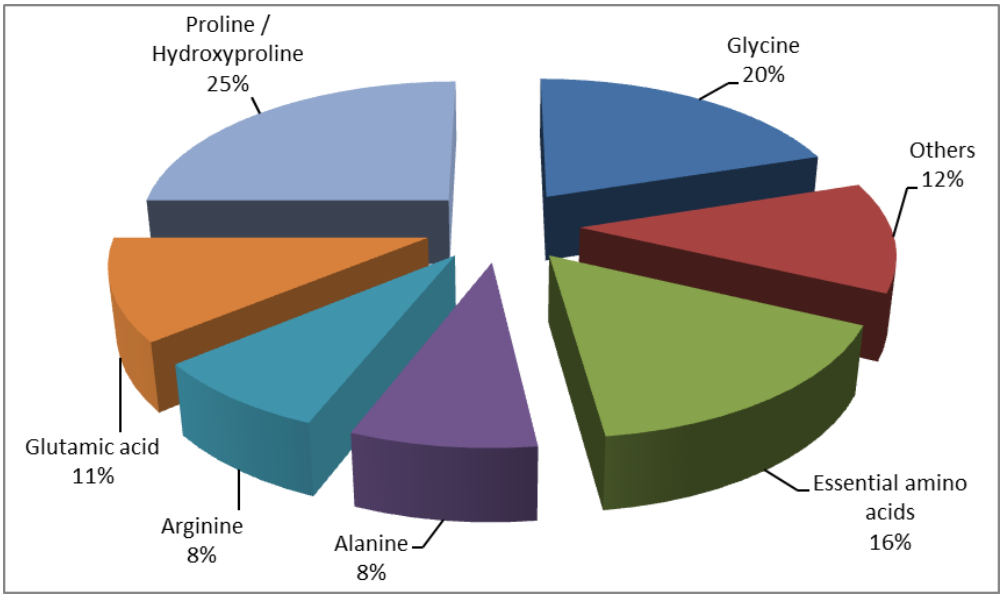

Fig. (6). Study conducted by Rousselot SAS, a French manufacturer of hydrolysed collagen (unpublished results).

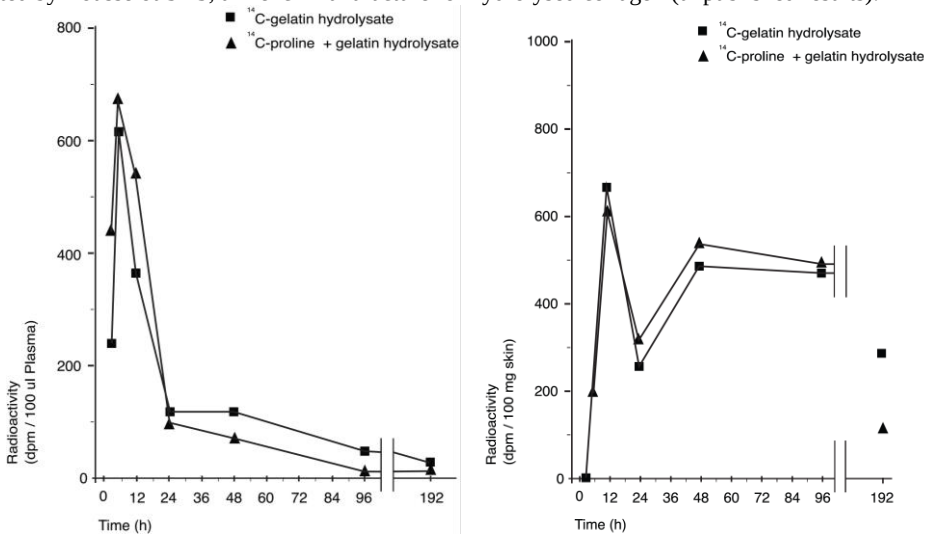

Fig. (7). Time course of radioactivity in plasma (left graph) and skin (right graph) of mice subsequent to the absorption of orally administered ^{14}C-labeled gelatin hydrolysate or of ^{14}C-labeled proline in the control group (Graph taken from Ref. [34]).

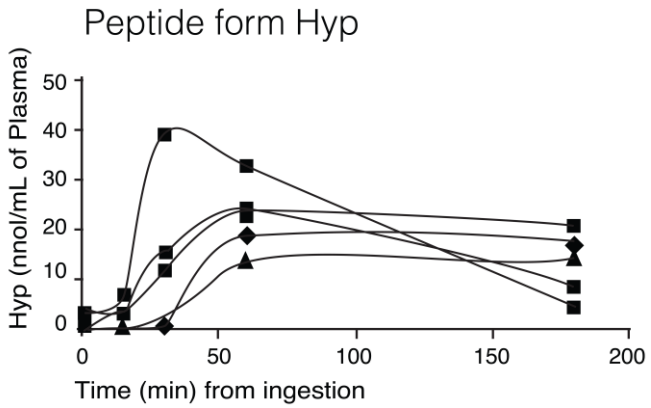

Fig. (8). Serum levels of hydroxyproline in peptide forms after oral ingestion of gelatin hydrolysate. (Graph taken from Ref. [35]).

Collagen and gelatin have long been used as a food source and/or supplement. Interestingly, collagen peptides have been shown to have an impact on weight loss and to be effective at maintaining weight, with no adverse effects (Rousselot – *Collagen peptides and nutrition*). The Food and Drug Administration (FDA) has classified gelatin, from which collagen peptides are prepared, as a safe substance. In addition, based on international research results, both the World Health Organization (WHO) and the European Commission for Health and Consumer Protection have confirmed that hydrolysed collagen is safe. Rarely minor side effects, such as nausea, flatulence or dyspepsia, may occur in some people by ingesting collagen peptides. In conclusion, the safety of collagen peptide and gelatin is widely recognized. (http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=scogslisting&id=141 http://www.gelatine.org/about-hydrolysed-collagen/safety.html http://www.efsa.europa.eu/en/efsajournal/doc/174.pdf).

DIGESTION AND ABSORBTION

In order to be active in the deeper layer of the skin, hydrolysed collagen must cross the intestinal barrier and reach the blood stream. It is worth noting that the rate of transport across the intestinal barrier could limit the efficacy of these compounds in the skin. Therefore, before speculating about their mechanism of action, it is important to demonstrate in what form and quantity collagen peptides can be absorbed.

According to Richelle *et al.* [33], bioavailability is defined as the relative amount of a dietary bioactive compound that crosses the intestinal barrier, reaches the blood circulation and is available for metabolic process or storage in the body (in this context, the skin).

Table 2. Scientific studies on the bioavailability of hydrolysed collagen.

Source	Hydrolyzed collagen concentration	Method	Results
Oesser *et al*. [34] Fig. (7)	10 mg of ^{14}C-labeled gelatin hydrolysate/g body weight	1) Testing on male mice: the test group received 10 mg of ^{14}C-labeled gelatin hydrolysate/g body weight, while the control group ^{14}C-labeled proline, together with unlabeled gelatin hydrolysate (10 mg/g body weight). 2) SDS-electrophoresis and HPLC to quantify the molecular weight distribution of the absorbed hydrolysed collagen.	1) 90% of orally administered hydrolysed collagen was absorbed within the first 12 hours from the intake. Radioactivity in skin reached its peak values 12 hours after the administration of ^{14}C-labeled hydrolysed collagen and remained relatively high up to 96 hours in contrast to plasma. 2) Hydrolysed collagen peptides within a range from 1 to 10 kDa were found on the serosal side of the intestine, indicating that collagen hydrolysate may be absorbed to some extent also in the high molecular form.
Iwai *et al*. [35] Fig. (8)	9.4–23 g of hydrolysed collagen.	Human volunteers ingested hydrolysed collagen from porcine skin, chicken feet, and cartilage after 12 hours of fasting.	1) Hydroxyproline-containing peptides increased in amount after collagen intake and reached a peak level after 2 hours till they decreased later on to half of the maximum level after 4 hours from the oral ingestion. 2) Identification of high levels of a small peptide proline-hydroxyproline (Pro-Hyp) found in the blood after hydrolysed collagen intake.
Ohara *et al*. [36]	Food-derived gelatin from three sources of type I collagen.	Five healthy male volunteers ingested, after 12 hours of fasting, type I gelatin hydrolysates from fish scale, fish skin, or porcine skin.	The aim of the study was to compare the quantity and structures of food-derived gelatin hydrolysates in human blood from three sources of type I collagen. Over a 24-hours period, amounts of Hyp containing peptides comprised around 30% of all detected Hyp. The total area under the concentration time curve of the fish scale group was significantly higher than that of the porcine skin group.

The first step of digestion consists in the degradation of hydrolysed collagen to form dipeptides and tripeptides or free amino acids. Several proteases (e.g. pancreatic proteases; small intestinal brush-border proteases; peptidase) are involved in the degradation process.

Several scientific studies done in animals and humans have described the bioavailability of hydrolysed collagen after oral administration and are summarized in Table **2**.

It is commonly assumed that prior to absorption, peptides are hydrolysed in the gastrointestinal tract, so predominantly the free amino acids enter the circulation [37, 38]. However, there is considerable evidence that peptides can also be absorbed. For example Hyp is absorbed in both amino acid (free form) and peptide form. Pro-Hyp is the major peptide found in human plasma after oral ingestion of any hydrolysed collagen.

The mechanism of absorption across the intestine membrane has been extensively studied. Epithelial cells are the principal site of absorption of several nutrients. There are three possible mechanisms for the transport of oligopeptides through the intestine: (i) PEPT1 mediated transcellular transport for di- and tripeptides [39], (ii) a transcytotic route known to be used for the transport of macromolecules (such as proteins) [40], and (iii) intracellular passive transport for the absorption of peptides [41]. However, the role of these pathways in intestinal oligopeptide absorption is not completely understood.

The trans-cellular transport of these peptides mediated across intestinal epithelial cells is a two-step mechanism, which involves the transport across two different membranes: i) peptide take up by the epithelial cell from the lumen across the brush-border membrane; ii) absorption into the blood stream across the basolateral membrane [42]. The first step is mediated by H^+-coupled peptide transporters (PEPT1 and PEPT2). In particular the transporter PEPT1 operates an enantioselective transport of neutral and mono or polyvalently charged peptides [43]. It has been shown that

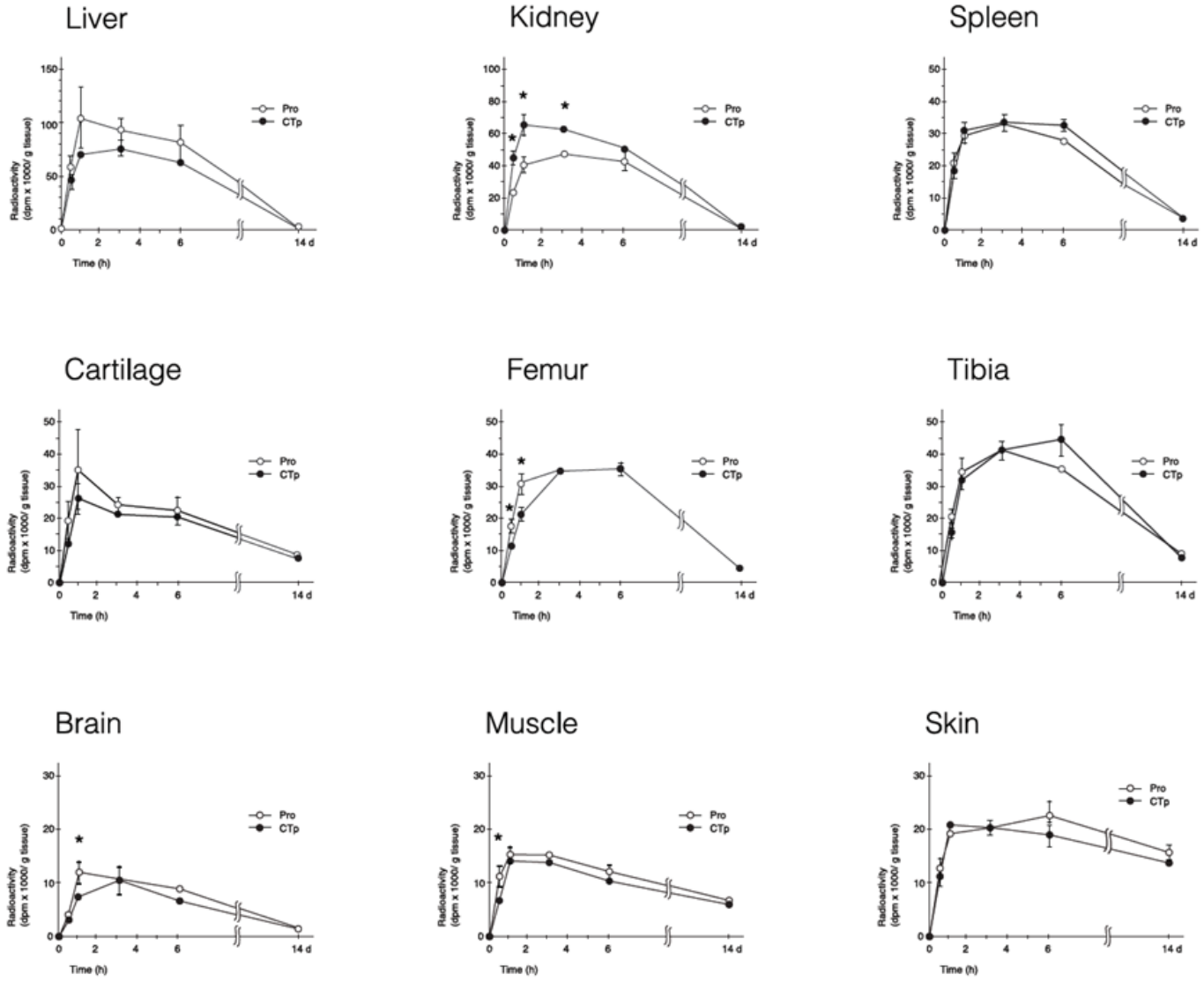

Fig. (9). Tissue distributions of radioactivity in rats administered with collagen peptides. (Graph taken from Ref. [32]).

collagen derived peptides (proline-hydroxyproline and glycine-proline-hydroxiproline) are absorbed through PEPT1 transporter [43].

DISTRIBUTION

Distribution is usually defined as the process by which a compound reaches the target tissue through the blood circulation. Factors that can affect the rate of distribution are the blood flow or chemical feature of a given compound such as molecular size or polarity.

Watanabe-Kamiyama *et al*. [32] studied the distribution of collagen peptides to the skin and other tissue by means of an *in vivo* experiment, where proline or collagen peptides ^{14}C-labeled were administered to Wistar rats. Radioactivity was measured in the different tissues after ingestion of the collagen peptides and/or proline during 0 to 6 hours and until 14 days (Fig. (**9**)). The results were very promising in terms of residence in the skin and showed that the radioactivity remains in this tissue at high levels up to 14 days. This indicates the ability of collagen peptides to reach the dermis in the skin where their main benefit is observed.

ACTION IN THE DERMIS

As previously described, several experiments have shown that collagen peptides can be efficiently absorbed and distributed to the deepest skin layers. However, this still does not represent a proof of their efficacy.

The final step is to understand whether collagen peptides can be active in the skin and what is the mechanism of action. Several *in vitro* and *in vivo* studies have proven the efficacy of these peptides, evidencing their ability to target fibroblasts. In the following Tables (Table **3** and Table **4**) are described some key studies that illustrate the ability of collagen peptides to: 1) stimulate the proliferation and motility of fibroblasts; 2) induce an increase in collagen fibres' density and diameter in the dermis; 3) increase hyaluronic acid production; 4) activate protection against UVA radiation.

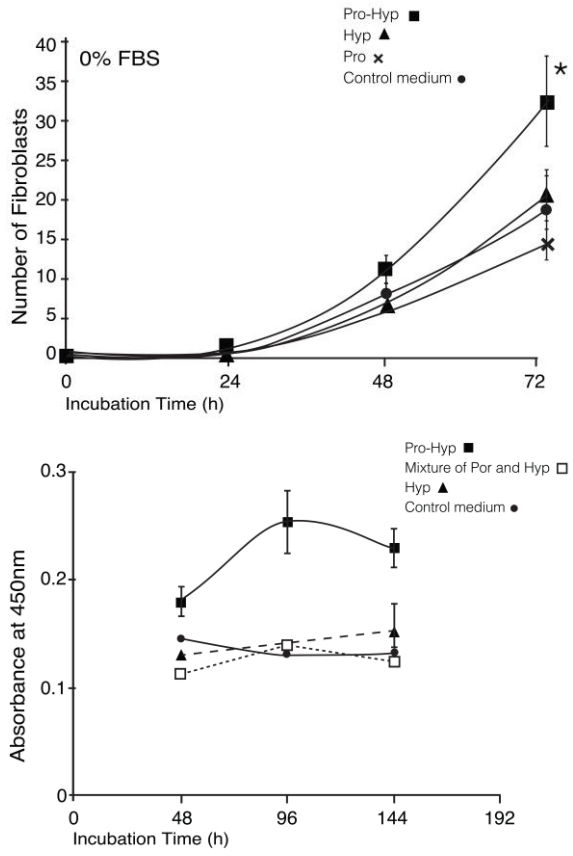

Fig. (10). Fibroblast migration (left graph) and proliferation (right graph) induced by Pro-Hyp (■), Hyp (▲), Pro (●) and the control medium (×). (Graph taken from ref. [45]).

CLINICAL TRIALS HAVE PROVEN THE EFFICACY OF HYDROLYSED COLLAGEN

Skin ageing is caused by several factors: ionizing

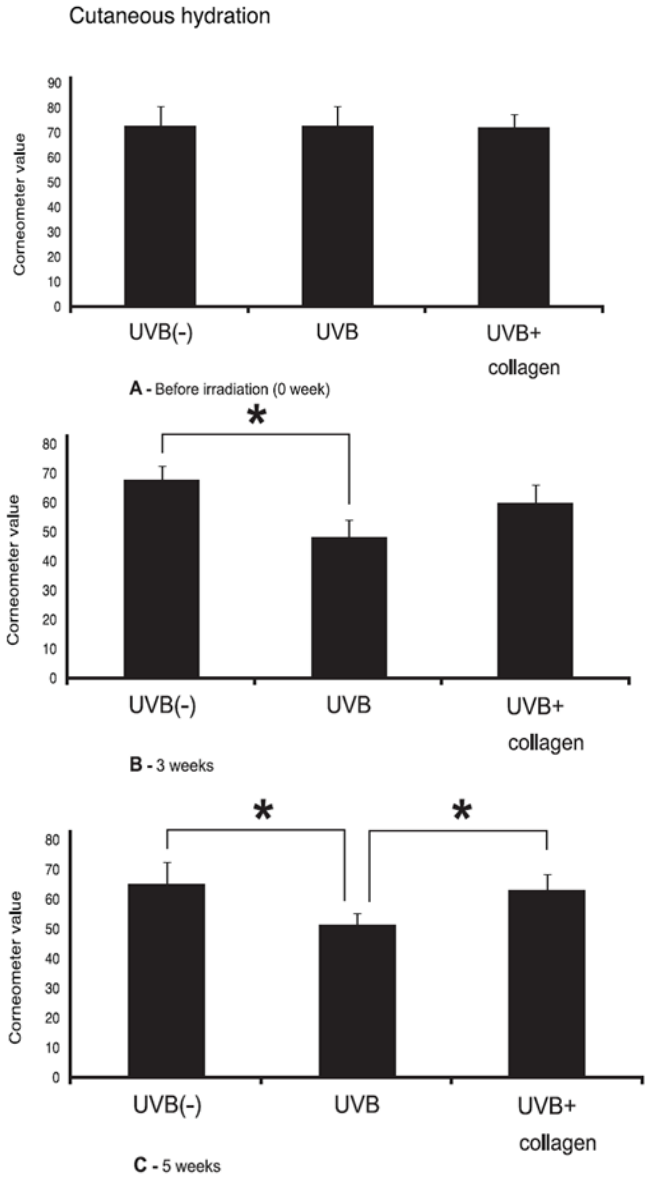

Fig. (11). Effects of UV-B irradiation and hydrolysed collagen on stratum corneum hydration from Ref [49].

radiation, physical and psychological stress, alcohol intake, poor nutrition, overeating, environmental pollution, and exposure to UV radiation [52]. In order to better study the different benefits of hydrolysed collagen on skin, controlled clinical trials are needed in addition to the previous preclinical and bioavailability assays. Up to date several clinical trials have been performed proving the efficacy of collagen peptides.

Two studies conducted by Rousselot SAS of France have investigated the effect of hydrolysed collagen on skin hydration and reduction of deep wrinkles. In the first study a double-blind, randomised, placebo-controlled clinical trial was carried out in Japan on 33 women aged 40-59 years with normal to dry skin. The results showed a 28% increase in skin hydration by taking 10g of hydrolysed collagen (Fig. (**12**)).

In the second study a double-blind, randomised, placebo-controlled clinical trial was carried out in France on 47 women aged 35-55 years with normal to dry skin. The positive outcome showed a 30% decrease in the formation of deep wrinkles, after supplementation of 10g of hydrolysed collagen (Fig. (**13**)).

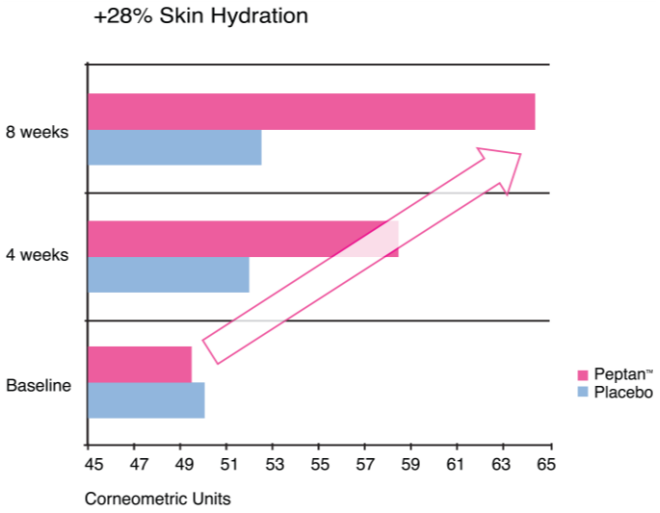

Fig. (12). Clinical trials on skin hydration. (Unpublished results by a study from Rousselot).

Other up to date clinical trials are shown in Table **6**.

Table 3. ***In vitro*** **scientific studies on the efficacy of collagen peptides.**

Source	Aim of the study	Results and main conclusions
Postlethwaite *et al*. [44]	To analyse the chemotactic response of human dermal fibroblasts to type I, II, and III human collagens and collagen derived peptides by an *in vitro* assay.	All three native human collagens and constituent α-chains serve as *in vitro* chemo-attractants for fibroblasts. In addition, also di- and tri-peptides containing hydroxyproline resulted chemotactic for fibroblasts. The authors suggested that both collagen and collagen derived peptides might function as chemotactic stimuli for fibroblasts *in vivo* and attract these cells for the repair of damaged tissues.
Shigemura *et al*. [45] Fig. (10)	To investigate the influence of collagen peptides on migration and growth of mouse skin fibroblasts.	The authors report that the number of cells migrating from the explanted skin increased significantly after treatment with Proline-hydroxyproline peptide. However they also found contrasting results and finally concluded that Pro-Hyp has a minor effect on fibroblasts' motility. On the other hand they showed that Pro-Hyp increases significantly fibroblasts growth.
Chen and co-workers [46]	To study the effect of different concentrations of hydrolysed collagen from fish on fibroblasts and keratinocytes. Investigation of both proliferation and collagen secretion and/or the expression of mRNA type I collagen.	A concentration of collagen ranging between 48~97 µg/mL resulted in an increase in proliferation percentage of 191%. Also the highest keratinocytes proliferation was achieved with a collagen concentration between 0.76~1.53 µg/mL and induced an increase in proliferation percentage of 242%. Finally the authors reported an increase in the expression of collagen I mRNA from fibroblasts.
Ohara *et al*. [47]	To support the function of collagen peptides in stimulating dermal fibroblasts proliferation and synthesis of hyaluronic acid.	In this study eight different collagen derived peptides, containing Hyp, were analysed. Positive effect on the proliferation was observed for Ala-Hyp, Ala-Hyp-Gly and Pro-Hyp. Pro-Hyp induced the maximal stimulation of cell proliferation of ~ 1.5 fold. Further investigation was performed on the effect of different concentrations of Pro-Hyp. In addition the same eight peptides were tested to study their effect on hyaluronic acid synthesis. The results were consistent with the proliferation study as Pro-Hyp showed highest efficacy, where 200 nmol/mL induced a 3.8 fold increase in hyaluronic acid synthesis. In addition the authors suggest that hyaluronic acid increases hydration of the extracellular space that aids fibroblast proliferation.

Table 4. ***In vivo*** **scientific studies on the efficacy of collagen peptides.**

Source	Aim of the study	Results and main conclusions
Minaguchi *et al.* [48]	To analyse the effects of two doses (0.2 g/kg and 1.0 g/kg body weight) of hydrolysed collagen ingestion on the extracellular matrix of the Achilles tendon of rabbit, for 56 days. The size of collagen fibrils and the amount of glycosaminoglycans were measured in comparison with those of a rabbit fed with a control protein (lactalbumin) or water alone.	Though an increase in collagen fibril diameter was observed with lactalbumin, collagen fibrils were much thicker when rabbits ingested collagen peptides. Statistical analysis revealed that while in the water group fibrils with a diameter between 20-60 nm had the highest probability, in presence of hydrolysed collagen the highest probability values were in the range 160-180 nm. Although these effects were observed in tendons, these results may support a benefit on skin, since in both the tissues type I collagen was the major component of extracellular matrix.
Matsuda *et al.* [2]	To examine, in the dermis of pig, the effects related to collagen peptide ingestion (0.2 g/Kg body weight, for 62 days) on fibroblasts and on the extracellular matrix (Table **5**).	The authors found a significant increase in collagen fibres density and diameter along with an increase in fibroblasts density. The diameter of collagen fibrils didn't change significantly between the control and animals that ingested the lactalbumin containing diet. Instead, the diameter and density of collagen fibrils increased significantly when collagen peptide was administered, and this was associated with an increase of the density of fibroblasts. This implies that the effect mediated by hydrolysed collagen was protein specific and not depending on amino acid intake.
Schibuya *et al.* [61]	To investigate the effects of the treatment with collagen peptides together with vitamin C in age-related skin pathology.	The study was done in hairless Sod1−/− double mutant mice and the authors showed that co-treatment with these compounds corrected age-related skin thinning by attenuating oxidative damage.
Tanaka *et al.* [49] **Fig. (11)**	To examine the effect of daily ingestion of collagen peptides on skin after damage induced by repeated UV-B irradiation.	The length of the study was 6 weeks and it was conducted on hairless mice divided in three groups: 1) mice not exposed to UV-B 2) mice exposed to UV-B; 3) mice exposed to UV-B but fed with collagen peptides 0.2 g/Kg body weight daily. After the 6 weeks period skin samples were taken and analysed. On mice exposed to UV-B irradiation a decrease was observed in the hydration of skin, hyperplasia of the epidermis occurred and there was a reduction in collagen I levels. On the other hand, ingestion of collagen peptides improved significantly skin condition and collagen levels. The authors suggested that collagen peptides, as a dietary supplement, are beneficial to suppress UV-B induced skin damage and photo-ageing.
Zhang *et al.* [50]	To investigate the effect of marine collagen peptides on wound healing and angiogenesis in rats.	96 animals were randomly treated with vehicle or with 2 g/kg marine collagen peptides. Wound closure and tensile strength were calculated. Angiogenesis was assessed by immunohistological methods. The rats treated with marine collagen peptides showed quicker wound closure and better tissue regeneration at the wound site. Moreover, marine collagen peptides treatment improved angiogenesis and contributed in forming a thicker and better organised collagen fibre deposition when compared to vehicle-treated group.
Liang *et al.* [51]	To investigate the long-term effects of Salmon skin marine collagen hydrolysate on the anomalous collagen matrix homeostasis in chronological aged skin.	4 weeks old rats were supplemented with oral intake of marine collagen hydrolysate (diet concentrations of 2.25% and 4.5%) for 24 months. The histological and biochemical analysis revealed that marine collagen hydrolysate inhibited the collagen loss and collagen fragmentation in chronological aged skin. Based on immunohistochemistry and western blot analysis, collagen type I and III protein expression levels significantly increased in marine collagen hydrolysate-treated groups when compared with the aged control group. Moreover, marine collagen hydrolysate could alleviate the oxidative stress in chronological aged skin due to its influence on collagen matrix homeostasis.

Table 5. Effects of collagen peptides ingestion on fibroblast and extracellular matrix.

	a) Control (fibrilser)	b) Lactalbumin	c) Collagen peptide
Density of fibroblast (cells/mm2)	33.3 ± 0.9	32.3 ± 0.7	40.2 ± 0.9
Diameter of collagen fibrils (nm)	103.2 ± 0.4	102.1 ± 0.5	106.4 ± 0.5
Diameter of collagen fibrils (fibrils/m m2)	77.9 ± 2.7	74.3 ± 2.2	90.5 ± 1.8

Full data are shown in Ref. [2]

Table 6. Hydrolysed collagen clinical trials.

Source	Aim of the study	Results and main conclusions
Sumida *et al*. [53]	To evaluate the effect of daily ingestion of hydrolysed collagen (10 g) on skin hydration of 20 healthy Japanese women and compare this to placebo group (19 volunteers).	Through 60 days, a gradual improvement in water absorption capacity was observed in volunteers who ingested collagen peptides (when compared to placebo group). However, this improvement was not statistically significant between the treated group and the placebo. This could be addressed to the low number of volunteers included in the trials.
Matsumoto *et al*. [54]	To evaluate if daily ingestion of collagen peptides improves skin hydration.	The authors found an improvement of the skin condition of women volunteers after ingestion of fish collagen peptide for 6 weeks. The percentage of positive response between the subjects was very high, however the study did not have a placebo control.
Matsumoto *et al*. [55]	To evaluate if daily ingestion of collagen peptides improves skin hydration.	In this study 2.5, 5 and 10 g of fish collagen peptide were administered and compared to the placebo. The skin stratum corneum hydration was measured at baseline and after 4 weeks. When all subjects were included in the analysis no significant difference between the treated groups (2.5/5/10 g) and the placebo was observed. However when only the subjects older than 30 years were considered, there was a significant difference ($P<0.05$) between the treated group (5 and 10 g) and the placebo.
Koyama *et al*. [56]	To evaluate if ingestion of collagen peptides improves skin condition.	This study demonstrated that women after ingestion of 5 or 10 g of pig skin collagen perceived improvement of their skin already after 3 weeks and at the end of the treatment after 7 weeks.
Proksch *et al*. [57]	To investigate the effects of collagen hydrolysate on skin biophysical parameters related to cutaneous ageing: skin elasticity, skin moisture, transepidermal water loss and skin roughness.	In this study 69 women (35-55 years old) were randomized to receive collagen hydrolysate (2.5 g or 5.0 g) or placebo once a day for 8 weeks. At the end of the study, skin elasticity in both collagen hydrolysate dosage groups showed a statistically significant improvement when compared to placebo. In terms of skin moisture and skin evaporation, a positive influence of collagen hydrolysate treatment could be observed in a subgroup analysis, but data were not significant.
Schwartz and Park [58]	To investigate the effect of a dietary supplement, containing hydrolysed collagen type II, hyaluronic acid and chondroitin sulfate, in 26 healthy females with signs of natural photo-ageing in the face.	Daily supplementation with 1 g of hydrolysed collagen for 12 weeks brought to a significant reduction ($P<0.05$) of skin dryness/scaling and global lines/wrinkles. Moreover, a significant increase in hemoglobin and collagen content of the skin dermis was observed after 6 weeks of supplementation. At the end of the study, the hemoglobin increase remained significant, while the increase in collagen content was maintained, although the difference from baseline was not significant. The authors suggested that dietary supplementation with hydrolysed collagen can physiologically counteract natural and photo-ageing processes. A placebo controlled study is necessary to confirm these observations.

Table 6. Contd…..

Source	Aim of the study	Results and main conclusions
Béguin [59]	To test the efficacy and safety in skin ageing of a micronutrient supplement, containing marine collagen proteins, through a 4 month randomized double-blind controlled clinical study.	The trial included 40 subjects. The supplement was tested against placebo for a period of 3 months followed by 1 month without supplementation to assess lasting effects. Efficacy measurements were: skin surface evaluation, ultrasound measurement of sun-exposed skin and protected areas and photographic assessment. When compared to placebo, all investigated parameters showed a continuous and significant improvement in the group taking the supplement during the 3 months of trial ($P<0.01$). Photographs showed visible improvement of the overall skin appearance and a reduction of fine lines. In the active group, ultrasound measurements showed an increase in dermis density of up to 78%. The final assessment, after 1 month without supplementation, showed no further improvements and there was a slight decrease in most improved parameters. No treatment-related side effects were reported. The study demonstrated that the supplement may be effective to protect the skin and support its repair process.
Choi *et al*. [60]	To evaluate the effect of daily collagen peptide supplementation on skin properties.	In a randomized trial, 32 healthy volunteers received for 12 weeks either: - no supplement - collagen peptide 3 g - collagen peptide 3 g and vitamin C 500 mg - vitamin C 500 mg Skin properties such as hydration, transepidermal water loss and elasticity were evaluated. The data showed that daily collagen peptide supplementation improved skin hydration and elasticity ($P<0.05$), but the association with low-dose vitamin C intake did not enhance the effect of collagen peptide on skin properties.

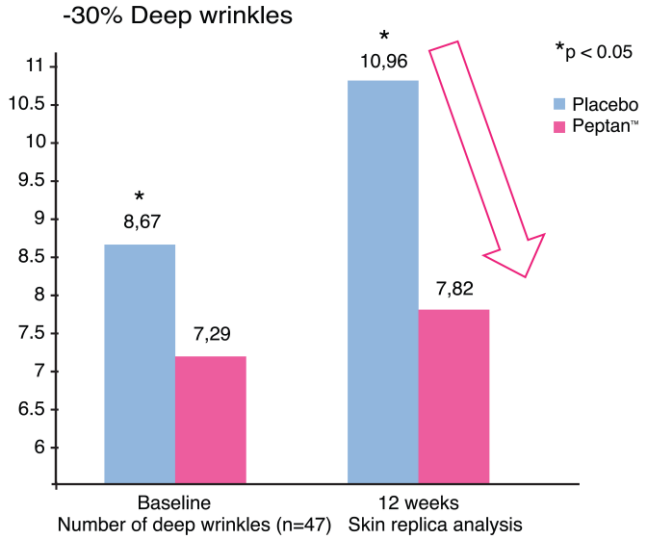

Fig. (13). Clinical trial on reduction of deep wrinkles. (Unpublished results by a study from Rousselot).

THERAPEUTIC IMPLICATIONS

The evidence supporting the efficacy of hydrolysed collagen in treating the many sequelae of skin ageing (wrinkles, skin thickening etc.) has led to the development of a number of functional foods (food containing additives which provide extra nutritional value) also known as nutraceuticals which contain hydrolysed collagen. Various studies are ongoing to prove the clinical efficacy and safety of these promising new products.

CONCLUSION

The skin is the largest organ of the human body and represents the main barrier to the external environment. Collagen, elastin and hyaluronic acid are the skin main components and have an important role in maintaining its structure and hydration.

The collagen family consists of 28 different proteins, which account for 25% - 35% of the total protein mass in mammals. Type I collagen is the most abundant in human skin (80%) with type III collagen making up the remainder of skin collagen (15%). The collagen in the skin is mainly produced by fibroblasts.

Hydrolysed collagen consists of small peptides with low molecular weight, enriched in specific amino acids: glycine, proline and hydroxyproline. Due to its low molecular weight, hydrolysed collagen is highly digestible, absorbed and distributed in the different tissues of the human body. Several experiments have shown that collagen peptides can be efficiently absorbed and distributed to the dermis, the deepest layer of the skin, where they can stimulate the proliferation and motility of fibroblasts; induce an increase in the density and diameter of collagen fibres; increase hyaluronic acid production and activate protection against UVA radiation.

To date several controlled clinical trials have been performed proving the efficacy and benefits of collagen peptides on skin properties, such as hydration, elasticity and reduction of wrinkles and recently a number of therapeutic formulations of hydrolysed collagen have been developed for the treatment of skin ageing. These fall into the category of nutraceuticals – products offering real benefits to all those with ageing skin.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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