



Collagen Peptide

Research Reports

Ingestion of collagen Peptide

Collagen report No.1

Gelatin has long been used in both foods and medicine. Collagen peptide, which is made by decomposing gelatin with proteinase, is now increasingly being used as a supplement. Here, we briefly review studies, including our own, on the effects of ingesting collagen peptide.



1) COLLAGEN, GELATIN AND COLLAGEN, PEPTIDE

"Collagen" is the most abundant protein in our body, comprising about one third of total protein. The molecular size of collagen is 300 kDa, as collagen is composed of three polypeptide chains, each of which is 100 kDa.

"Gelatin" is made industrially by extracting heated natured collagen from bone or skin in boiling water. The three polypeptide chains of the collagen molecule are dissociated by heating, and hydrolysis of each polypeptide chain occurs. Therefore, the molecular size of gelatin is 100 kDa or less, although considerable variations in size are observed. Gelatin is soluble in hot water but forms a gel when dissolved in cold water.

"Collagen peptide" is prepared by decomposing gelatin into smaller sizes using proteinase. Collagen peptide does not form a gel and readily dissolves in cold water, even at relatively high concentrations. Thus, collagen peptide is easier to ingest in large amounts than native collagen or gelatin. The molecular size of collagen peptide varies widely (0.3~8 kDa) depending on the method and conditions of decomposition.

Although native collagen, gelatin and collagen peptide each represent different forms of the collagen molecule, they are often collectively referred to as 'collagen' when used in supplements, cosmetics or food.

2) EFFECTS ON BONE

If the amount of daily intake of protein is low, bone mineral density decreases. Low protein conditions may be a cause of lower bone mineral density in the elderly. We performed an animal experiment to investigate the effects of collagen ingestion on low protein conditions (1). When mice are given food containing 10% casein, bone mineral density becomes significantly lower when compared to mice given food containing 14%. Figure 1 shows bone mineral density of mice raised for 10 weeks with food containing 6% casein, 10% casein or 6% casein + 4% collagen. The horizontal axis shows the position in the femur, while the vertical axis indicates the bone mineral density at that position. Bone mineral density in the mice given food containing 6% casein + 4% collagen was significantly higher when compared to mice given 10% casein. It is known that bone mineral density increases with body weight. However, in this study, no significant difference in body weight was observed. These results thus suggest that collagen is superior to casein in its potential to enhance bone mineral density during protein under nutrition. Wu et al. (2) and Nomura et al. (3) confirmed the beneficial effects of collagen ingestion on bone mineral density and mechanical strength.

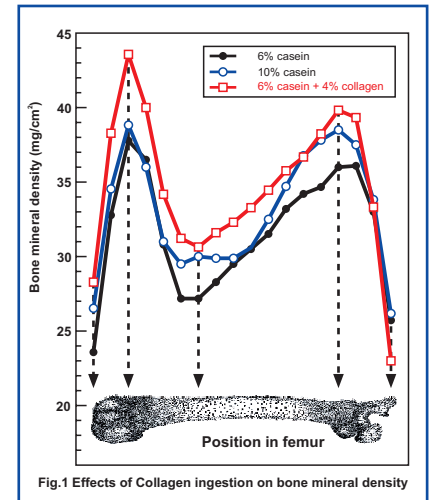


Fig.1 Effects of Collagen ingestion on bone mineral density

3) EFFECTS ON SKIN

We carried out a double blind test in order to examine the effects of collagen intake on skin hydration (4). Young female volunteers consumed a drink containing 10 g of collagen or placebo every day for 2 months. Figure 2 shows the increase in water absorbing capacity of the stratum corneum of the skin during the test period. The increase in water absorbing capacity of the collagen-ingesting group (80.2 S) was larger than that of the placebo group (10.8 S) at 1 month, and the difference became larger between the collagen group (203.5 S) and the placebo group (113.7 S) when the test period was extended to 2 months. The increase in the placebo group may be explained by the fact that both the placebo drink and the collagen drink contained vitamin C, which may have improved skin function. Morganti et al. (5) also reported that skin hydration increased when a patient with dry skin ingested collagen.

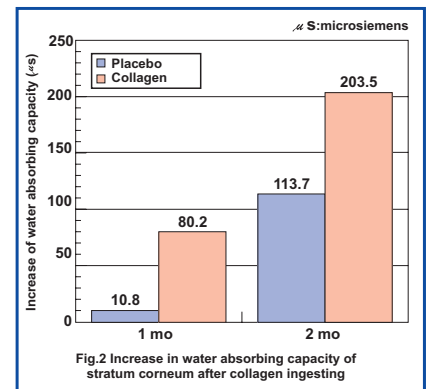


Fig.2 Increase in water absorbing capacity of stratum corneum after collagen ingesting

4) EFFECTS ON NAIL AND HAIR

Nail defects are a common complaint among women. Rosenberg et al. (6) showed that ingestion of collagen improved nail defects; they administered 7 g of collagen daily and found that nail defects were improved in 43 of 50 patients (86%) (Fig. 3). It was also reported that the cessation of collagen ingestion resulted in the reappearance of nail defects. Schwimmer et al. (7) also reported improvements in nail defects in 80% of patients after ingestion of collagen peptide. The main component of hair is keratin, as is the case for stratum corneum and nails. Scala et al. (8) investigated the effects of collagen ingestion on growth and thickness of hair. They found that the thickness of hair increased significantly after collagen ingestion for 62 days, and more pulling force was required to break the thickened hair. The increase in hair thickness was more evident in women than in men, possibly because the initial thickness was smaller in women. Hair thickness returned to initial size when collagen ingestion stopped.

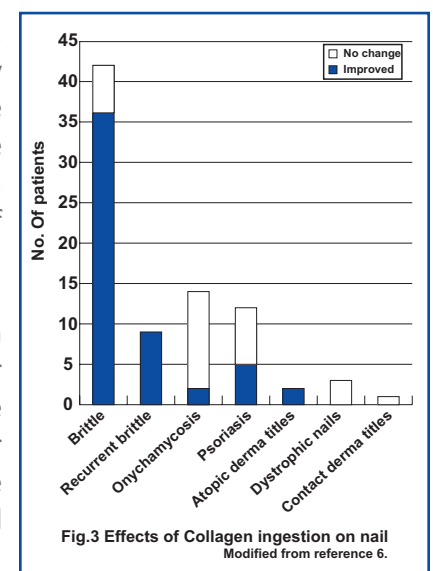
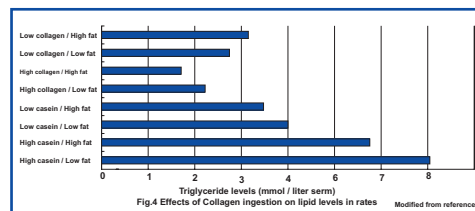


Fig.3 Effects of Collagen ingestion on nail
Modified from reference 6.

5) EFFECTS ON TRIGLYCERIDE IN THE BLOOD

High concentrations of triglyceride in the blood cause arteriosclerosis. There are several reports suggesting that ingestion of collagen reduces triglyceride levels in blood. Ratnayake et al. (9) prepared 8 types of food for rats by combining high or low concentrations of collagen and casein with high or low amounts of lipid, as shown in Figure 4. It was found that triglyceride levels in blood decreased when rats were given collagen containing food than casein-containing food. Oliveira et al. (10) and Wu et al. (2) also reported the beneficial effects of collagen ingestion in reducing the amount of triglyceride in blood.



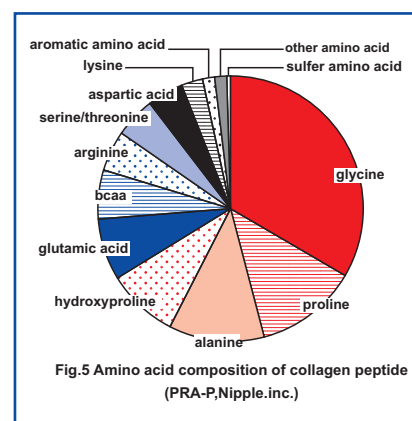
6) INGESTED COLLAGEN AS A SOURCE OF AMINO ACID

Figure 5 shows the amino acid composition of collagen made from porcine hide (PRA-P, Nippi Inc.). The amino acid composition is almost the same as native collagen. About one third of the amino acid in collagen is glycine, as collagen is largely comprised of Gly-X-Y amino acid sequences. Collagen also contains relatively large amounts of proline and hydroxyproline.

Glycine is a simple amino acid that plays various roles, including functioning as a neurotransmitter. Glycine is also a precursor for synthesis of heme and glutathione, as well as proteins, in the body. Although glycine is not an essential amino acid, it is important to consume sufficient amounts on a daily basis. The ratio of branched carbon amino acids (BCAA; valine, leucine, isoleucine) to aromatic amino acids (phenylalanine, tyrosine) is relatively high in collagen.

Therefore, collagen is used clinically to improve the amino acid imbalances in liver cirrhosis.

Free amino acid has a unique taste, which may lead to difficulties when added directly to foods. In contrast, collagen is tasteless and odorless, and thus has the least effect on food flavor. The effects of collagen ingestion may be partially explained by the function of amino acids contained in collagen. However, the beneficial effects of collagen ingestion described cannot be explained solely based on the function of free amino acid. A mechanism specific to collagen digestion and absorption is probably involved in the effects of collagen ingestion (11).



7) SAFETY OF COLLAGEN INGESTION

Supplement safety is far more important than effectiveness. Collagen has long been used all over the world in foods and medicine, indicating that collagen is a highly safe material. We carried out biochemical analyses of blood in the human clinical test described above (4), in which 10 g of collagen was taken every day for 2 months. No abnormal changes were observed in any of the 23 items examined.

Moskowiz (12) examined the adverse effects of collagen ingestion (10 g/day) for 24 weeks. In this study, minimal adverse effects, typically gastrointestinal complaints characterized by fullness or unpleasant taste, were observed, but no severe adverse effects were noted. In an animal study, Wu et al. (2) also reported that collagen ingestion provided beneficial effects on bone metabolism without obvious undesirable effects. Thus, ingestion of appropriate amounts of collagen is considered safe.

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Ingestion of collagen Peptide

Collagen report No.2

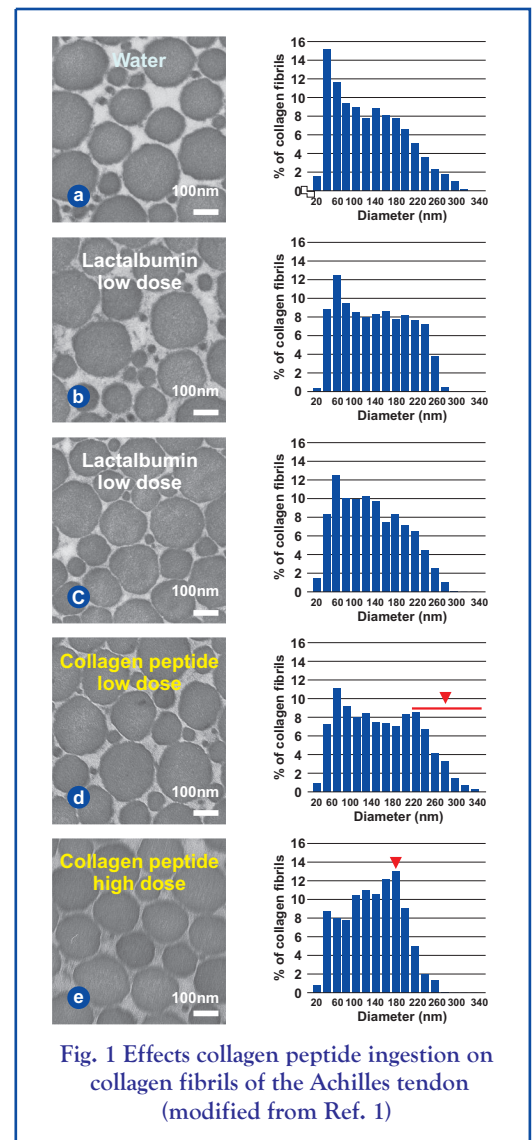
Collagen peptide for supplements is produced through the partial hydrolysis of collagen. Its market in Japan has increased rapidly in recent years. One reason for this rapid expansion has been the development of high-quality products with minimal odors and flavors, allowing the ingestion of amounts sufficient to realize actual effects on health and beauty. In this report, scientific studies performed by Nippi on the effects of collagen peptide ingestion on the Achilles tendon and the skin dermis are described. Furthermore, a method for estimating the amount of collagen in the diet is presented.

1) EFFECTS ON ACHILLES TENDON

Tendons are made of arranged collagen fibrils that connect muscle with bone. Excess exercise or awkward movements in sporting activities often result in injuries to the Achilles tendon, as well as nearby muscles, and can impair sporting performance. Therefore, it is essential for athletes to keep this tendon healthy.

Nippi has performed a scientific study on the effects of collagen peptide ingestion on the Achilles tendon. Nippi collagen peptide (molecular weight, 3,000~5,000 Da) was administered to rabbits at a dose of 0.2 g/kg body weight (BW)/day or 1.0 g/kg BW/day for 56 days and the collagen fibrils of the Achilles tendon were observed by transmission electron microscopy. Control animals received lactalbumin or water alone.

In the Achilles tendon of rabbits that ingested water alone, collagen fibrils of various diameters were observed (Fig. 1a). The graph on the right side shows the frequency (%) of collagen fibrils of the diameters indicated under the x axis. It was shown that collagen fibrils became thicker by ingesting lactalbumin, as shown in Figs. 1b and 1c. However, collagen fibrils were much thicker when rabbits ingested collagen peptide than when they ingested lactalbumin (Figs. 1d, 1e). This suggests that collagen fibrils in the Achilles tendon become thicker specifically in response to ingestion of collagen peptide (Ref. 1)



2) EFFECTS ON DERMIS

The dermis of the skin, like the Achilles tendon, contains numerous collagen fibrils; Collagen content in the dermis is about 90% of total protein. Elderly people, as well as younger individuals, have substantial interest in maintaining healthy and beautiful skin, and such skin is supported by the collagen-rich, deeper compartment of the skin (the dermis).

Nippi previously investigated the effects of ingestion of Nippi collagen peptide (molecular weight, 3,000~5,000 Da) on the collagen fibrils of the dermis. Collagen peptide or lactalbumin was added to the diet of pigs such that they were ingesting a dose of 0.2 g/kg BW/day for 62 days. The skin of the prescapula region of the neck was subjected to observation of collagen fibrils by transmission electron microscopy. The density of collagen-producing fibroblasts in the dermis was also determined.

The diameter of collagen fibrils did not differ significantly between animals that ingested normal diet and animals that ingested the lactalbumin-containing diet (Figs. 2a, 2b). However, 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 (Fig. 2c; Table 1). As was the case with the Achilles tendon described above, these results suggest that ingestion of collagen peptide has beneficial effects that are not found with other types of protein lactalbumin (Ref. 2).

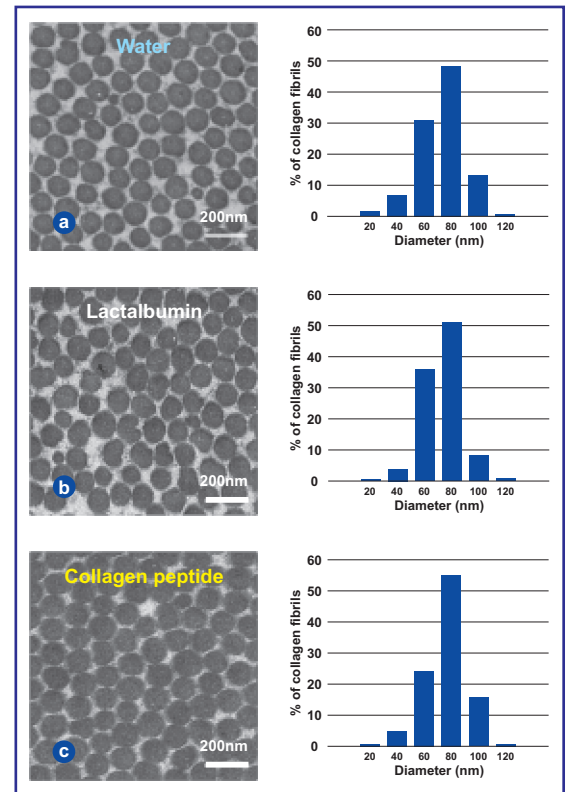


Table 1. Effects of collagen peptide ingestion on collagen fibrils in the dermis (modified from Ref. 2)

Item	a Control (water)	b Lactalbumin	c Collagen peptide
Thickness of the dermis (mm)	2.4 ± 0.2	2.3 ± 0.2	2.5 ± 0.2
Density of fibroblast (cells / mm ²)	33.3 ± 0.9	32.2 ± 0.7	40.2 ± 0.9 * #
Diameter of collagen fibril (nm)	103.2 ± 0.4	102.1 ± 0.5	106.4 ± 0.5 * #
Density of collagen fibril (fibrils / μm ²)	77.9 ± 2.7	74.3 ± 2.2	90.5 ± 1.8 * #

Significantly different from the control (water) group # Significantly different from the lactalbumin group

3) MECHANISM OF COLLAGEN PEPTIDE FUNCTION

It is known that ingested protein is digested and absorbed as amino acids. However, collagen peptide and gelatin are digested and absorbed in peptide form in part (Ref. 3,4). In 2005, Iwai et al. reported that prolylhydroxyproline, a dipeptide consisting of proline and hydroxyproline (ProHyp), was the main component of the peptide appearing in the body after ingestion of collagen peptide (Ref. 4). To elucidate the possible mechanisms for the beneficial effects of collagen peptide ingestion, the physiological activity of Pro-Hyp is currently under study.

Glycine comprises about one-third of the total amino acid in collagen peptide. Serum concentration of glycine increases transiently after ingestion of collagen peptide. It has also been reported that glycine has a number of physiological activities. Thus, ingestion of collagen peptide may affect our health and beauty through collagen-specific action mechanisms

4) COLLAGEN INTEKE FROM DIET

Collagen is commonly found in animal-derived foods. However, it is difficult to determine how much collagen is ingested in the course of daily life, as various animal-derived materials are present in the diet. Nippi has reported an easy method for estimating the amount of collagen in our diets (Ref. 5).

Nippi analyzed the amino acid composition of purified collagen from cattle, swine, chicken, fish and shellfish (Ref. 5). From these data, the amount of hydroxyproline in collagen, the “hydroxyproline factor”, was calculated. The amount of hydroxyproline in food was also determined by the dimethylaminobenzaldehyde method. Collage contents in food are calculated as follows: Collagen (mg/g) = (hydroxyproline factor)×(hydroxyproline) (mg/g), and calculated collagen contents are listed in Table 2. Table 3 shows the diet of an adult Japanese female. If she does not eat fish skin, the amount of collagen ingested is about 2.0 g/day, but this increases to about 3.1 g/day if she also eats fish skin. Thus, the amount of collagen ingested in daily life varies substantially with diet. Collagen thus represents an efficient supplement when daily intake of collagen is insufficient to maintain good health and beauty.

Table 1. Collagen contained in animal material. (Ref.5)

Meat/Fish food products	Notes	Collagen content (mg/g)
Beef		7.5
Beef tendon		49.8
Pork		11.9
Pork chitterlings		30.8
Chicken thigh		15.6
Chicken wing tip		15.5
Chicken drumette		19.9
Chicken liver		8.6
Chicken gizzard		23.2
Chicken stemum cartilage		40
Ham		11.2
Tuna		5.7
Salmon	Skinless	8.2
	With Skin	24.2
Sierra	Skinless	10.4
	With Skin	12.8
Yellowtail	Skinless	9.7
	With Skin	16.2
Broiled eel		55.3
Dagger tooth pike conger skin		76.6
Squid		13.8
Shrimp		11.5
Boiled and dried baby saridines		19.2
Sand eel		12.9
Short-neck clam		11.0
Oyster		9.8

Table 2. Collagen contained in diet

	Dish	Ingredients	Weight (g)	Amount of collagen (mg/g) Skinless	Amount of collagen (mg/g) with Skin
Breakfast	Wheat noodles topped with battered prawn	Wheat noodle	250		
		Fleshy prawn	25	275	288
		Long onion	5		
		Soft flour	7		
		Chicken egg	10		
		Vegetable oil	5		
		Bonito broth	300	180	180
		Superfine sugar	3		
		Dark soy sauce	24		
		Cooking rice wine	12		
Lunch	Pan-fried noodles	Pork	32	381	381
		Cabbage	48		
		Mung bean sprouts	48		
		Onion	20		
		Carrot	12		
		Vegetable Oil	3.2		
		Steamed chines noodles	120		
		High-quality rice wine	6		
		Worcestershire sauce	12		
		Green lever	0.4		
Lunch	Octopus dumplings	Chicken eggs	25		
		Soft flour	50		
		Octopus	20	276	276
		Green lever	1		
		Dried bonito flakes	1	17	17
		Worcestershire sauce	8		
		Ketchup	6		
		Vegetable oil	2		
		Soft flour	7		
		Chicken eggs	2.4		
Lunch	Corn croquettes	Breadcrumbs	5		
		Vegetable oil	5		
		Whole Milk	41		
		Butter	3.3		
		Salt	0.2		
		Pepper	0.06		
		Soup stock cube	1		
		Sweet corn	15		
		Worcestershire sauce	5		
		Dinner	Steamed rice	White rice	150
Salted salmon	70			574	1687
Pickled vegetable with squid	Squid / cuttlefish		2.5	35	35
	Herring		12.5	5	5
	Rishiri kelp		3.2		
	Carrot		3.5		
	Dark soy souce		6.5		
	Cooking rice wine		3		
	Refined rice wine		3		
	Chicken liver		30	258	258
	Ginger	2			
	Refined rice wine	6			
Liver boiled with ginger and soy sauce	Dark soy sauce	7.5			
	Superfine suger	1.5			
Total energy = 1.756kcal				Total 2,001	Total 3,125

5) SAFETY OF COLLAGEN PEPTIDE INGESTION

Collagen and gelatin have long been used as a food source and/or additive. Although minor side effects, such as nausea, flatulence or dyspepsia, may occur in some people by ingesting collagen peptide (Ref. 6), the U.S. Food and Drug Administration (FDA) has classified gelatin, from which collagen peptide is prepared, as a substance that is “generally recognized as safe” (GRAS). Furthermore, the Food and Agriculture Organization (FAO) and World Health Organization (WHO) have reported that there is no need to specify a limit for the daily intake of collagen. Thus, the safety of collagen peptide and gelatin is widely recognized

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Ingestion of collagen Peptide

Collagen report No.3

Collagen peptide is a popular supplement in Japan, and the market is currently growing rapidly, partly because collagen has documented effects on health and beauty. The mechanisms responsible for the effects of ingested collagen peptide in the body have long been unknown. However, identification of collagen-specific digested oligopeptides in the blood has made it possible to understand how collagen peptide exerts some of its beneficial effects. In this report, two scientific studies performed by Nippi on the effects of collagen peptide ingestion on the skin are described. Furthermore, up-to-date information on studies into the mechanisms of collagen peptide ingestion is also summarized.

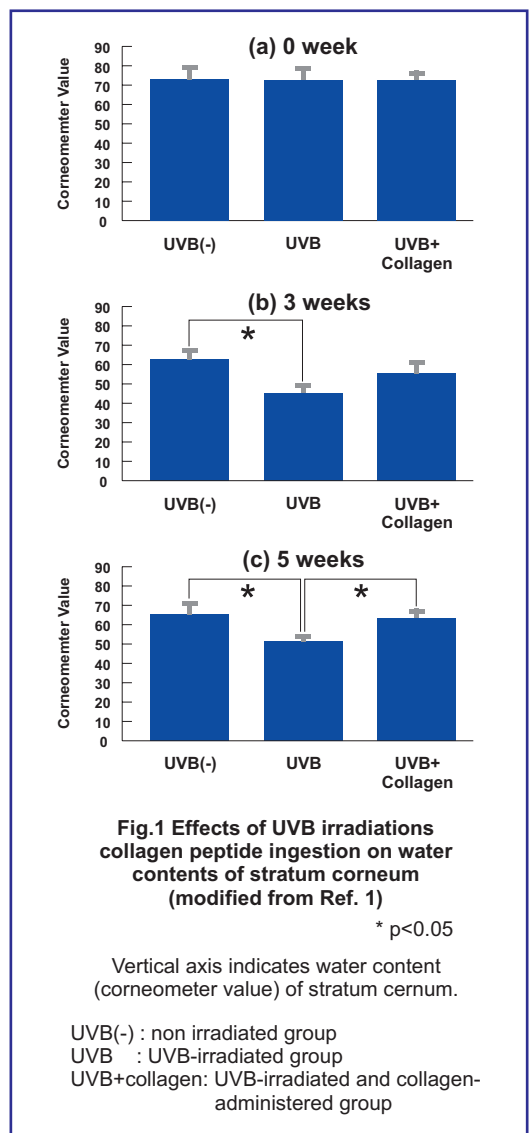
1) PREVENTION OF UVB - INDUCED SKIN DAMAGE

Ultraviolet light is divided into three categories depending on its wavelength: UVA (400~320 nm), UVB (320~290 nm) and UVC (290~200 nm). As UVC cannot penetrate the ozone layer, people on Earth are typically exposed to UVA and UVB. UVB has a higher energy and penetrates the epidermis to reach the upper layer of the dermis. In contrast, although the energy of UVA is lower than that of UVB, it reaches the deeper regions of the dermis.

Ultraviolet irradiation is a major cause of skin aging, and the incidence of skin cancer increases with exposure. Therefore, we studied the effects of collagen peptide ingestion on the skin damage induced by UVB irradiation in cooperation with researchers at the Tokyo University of Agriculture and Technology (Ref. 1).

It is known that repeated irradiation by UVB on the back skin of genetically hairless mice induces the formation of wrinkles. In our study, collagen peptide was administered to hairless mice at a dose of 0.2 g/kg body weight/day, and UVB irradiation was repeatedly performed at 0.3 mW/cm² for 6 weeks.

In order to assess skin function, water content of the stratum corneum was determined using a corneometer. The water Content of the stratum corneum decreased significantly after 3 weeks of UVB irradiation, thus suggesting that the skin is damaged by UVB. However, no significant decreases were observed in mice fed collagen peptide (Fig. 1a, b). Significant decreases in water content were also detected in UVB-irradiated mice at 5 weeks (Fig. 1c) and 6 weeks, but water contents remained significantly higher in UVB-irradiated, collagen peptide-fed mice than in UVB-irradiated mice without collagen peptide.



Hyperplasia of the epidermis is another form of skin damage induced by UVB. The epidermis in UVB-irradiated mice ($37.8 \pm 9.5 \mu\text{m}$, $p < 0.05$; Fig. 2b) was significantly thicker than that in nonirradiated mice ($27.1 \pm 5.9 \mu\text{m}$; Fig. 2a). However, ingestion of collagen peptide suppressed this skin damage, as the thickness of the epidermis in UVB-irradiated, collagen peptide-fed mice did not show significant hyperplasia ($31.1 \pm 5.6 \mu\text{m}$; Fig. 2c).

The dermis is also damaged by repeated UVB irradiation. Figure 3 shows the amount of type I collagen in the dermis, as detected with antibody against type I collagen. Type I collagen levels decreased clearly after UVB irradiation when compared with non-irradiated mice (Fig. 3, lanes 1 and 2). However, the amount of type I collagen did not decrease when collagen peptide was administered (Fig. 3, lane 3).

These results suggest that the daily ingestion of collagen peptide suppresses the skin damage induced by repeated UVB irradiation. It seems likely that the ingested collagen peptide is partly digested into small oligopeptides and absorbed into the blood, and that these oligopeptides suppress UVB damage or promote recovery after it, as it is unlikely that ingested collagen peptide or its digested products exert protective effects by absorbing UVB directly. Further studies on the protective mechanisms of collagen peptide ingestion will make it possible to prevent the UV-induced photoaging of the skin.

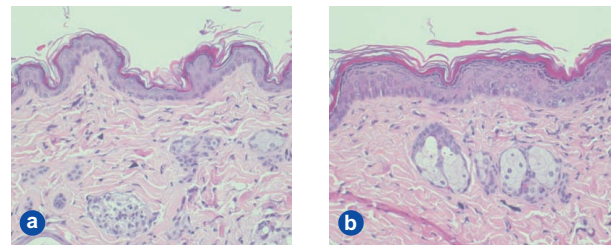


Fig. 2 Effect of UVB irradiation and collagen peptide ingestion on hyperplasia of the epidermis (modified from Ref. 1)

Skin sections were stained with hematoxyline and eosin.

- a** : non irradiated (UVB (-)) group
- b** : UVB-irradiated (UVB) group
- c** : UVB-irradiated and collagen administered (UVB+collagen) group

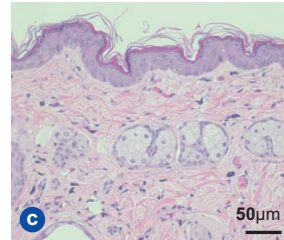


Fig. 3 Effect of UVB irradiation and collagen peptide ingestion on amount of type I collagen in the dermis (modified from Ref. 1)

Type I collagen in the dorsal skin at 6 weeks was visualized using antibody against type I collagen.

- Lane 1: non irradiated (UVB (-)) group
- Lane 2: UVB-irradiated (UVB) group
- Lane 3: UVB-irradiated and collagen administered (UVB+collagen) group

2) CLINICAL TEST OF THE EFFECTS OF THE HUMAN SKIN

The Japanese market for collagen peptide has been growing rapidly, as shown in Fig. 4; the market size in 2002 was ca. 700 t, but had increased to ca. 4,500 t in 2008 (data from Gelatin Manufacturers Association of Japan). However, the mechanisms of collagen peptide function remained unknown during this period. Therefore, it seems likely that the market grew rapidly because individuals can observe the effects of collagen peptide ingestion on health and beauty.

Nippi conducted a clinical test in 2009 in order to examine the effects of collagen peptide ingestion on the skin of healthy females aged 40~54 years (Ref. 2). In this placebo-controlled, double blind study, volunteers ingested a drink (50 ml) that did not contain collagen peptide (placebo drink), or

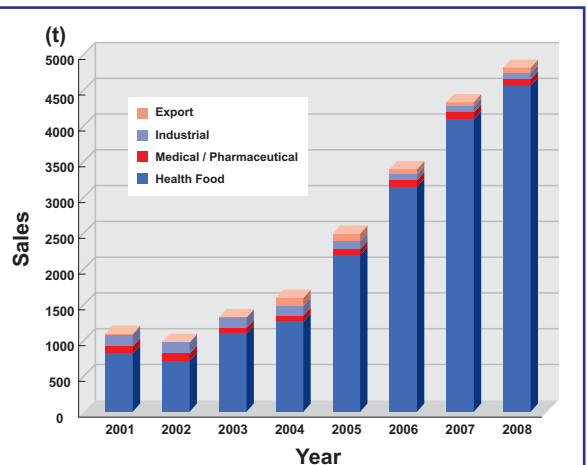


Fig.4 Development of collagen peptide market in Japan. (Data from gelatin Manufacturers of Japan.)

a drink that contained 5 g or 10 g of collagen peptide daily after dinner for 7 weeks, Without knowing which drink they were consuming. After 3 weeks and 7 weeks, a dermatologist asked the volunteers about their subjective impressions of facial skin condition (whether it was worse, normal or better as compared to before the test). The doctor was also blinded as to whether the volunteers had taken the placebo, 5-g collagen or 10-g collagen drinks (Fig. 5).

At 3 weeks, only 10% of the placebo group (n=20) answered “better”, while this value was 41% in the 5-g collagen peptide group (n=22; p<0.05) and 62% in the 10-g collagen peptide group (n=22; p<0.05). After 7 weeks, the value was 20% in the placebo group (n=20), but 81% in the 5-g group (n=21; p<0.05) and 74% in the 10-g group (n=19; p<0.05). These results confirm that 70~80% of woman are able to observe the effects of collagen peptide ingestion on the skin.

The next question to be addressed is how subjects realized that skin condition had improved. In the present study, both the water content of the stratum corneum and skin barrier function were determined. However, no clear differences were detected between the placebo group and the collagen peptide groups. These results suggest that ingestion of collagen peptide improves the function of deeper regions of the skin rather than function of the outermost epidermal regions.

Skin is not a simple organ. In the stratum spinosum of the epidermis, large numbers of bone marrow-derived immune cells (Langerhans cells; Fig. 6) are present. Epidermal cells secrete a variety of soluble proteins that control the functions of the skin and body. In the dermis, nerve bundles, nerve networks, blood vessels and lymphatic vessels are distributed in the extracellular matrix, whose main component is collagen (Fig. 7). Thus, it is important to determine which skin functions are improved by collagen peptide ingestion, and how these improvements lead to better facial skin condition

Medical examination by interview
 “How do you the condition of your face skin? Worth, **normal** or **better** compare to that before the test?”

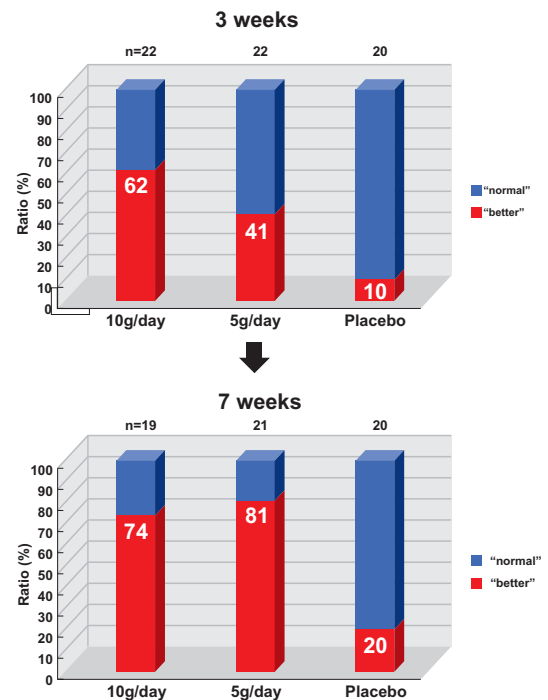


Fig. 5 Effects of collagen peptide ingestion on the skin (modified from Ref. 3)

Summary of questionnaire result obtained by a dermatologist at 3 week (upper) and 7 week (lower). Red: “better” ; Blue: “normal”

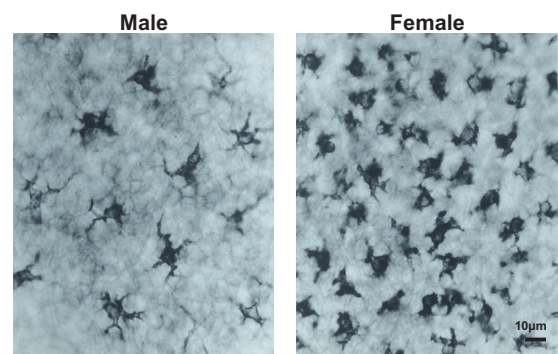


Fig. 6 Epidermal Langerhans cells (modified by Ref. 2)

ATPase staining of the mouse epidermis. Langerhans cells are distributed evenly in the stratum spinosum of the epidermis.

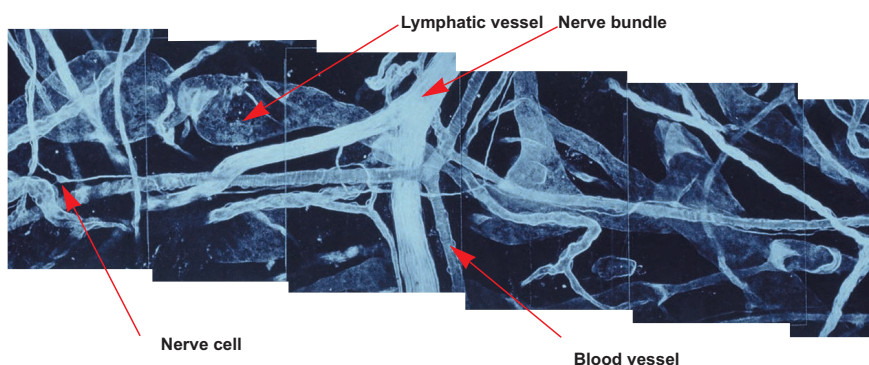


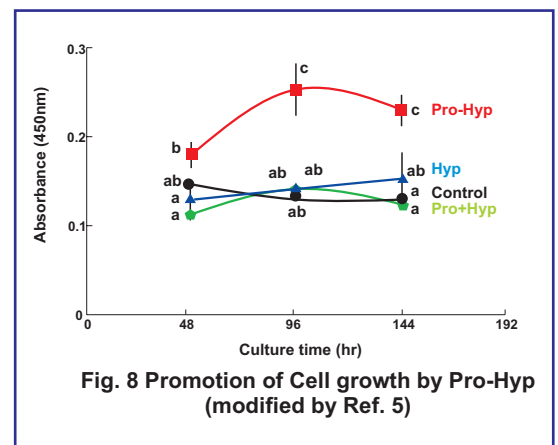
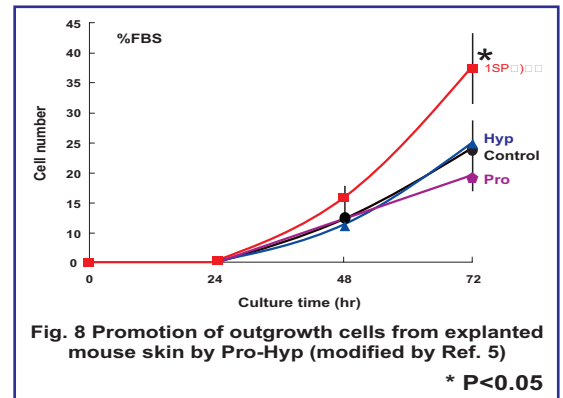
Fig. 7 Three dimension staining of dermal structures.

Dermise of mouse ear skin was stained using anti-laminin antibody and observed by confocal laser scanning microscopy.

3) MECHANISMS OF COLLAGEN PEPTIDE FUNCTION

Ingested protein is typically digested and absorbed as amino acids; however, collagen is partly absorbed in peptide form (Ref. 3). In 2005, Iwai et al. reported that prolylhydroxyproline (ProHyp), a dipeptide consisting of proline and hydroxyproline, was the main peptide component appearing in the body after collagen peptide ingestion (Ref. 4). In 2009, Shigemura et al. (Ref. 5) reported that the number of cells spreading from explanted mouse skin increased significantly after treatment with Pro-Hyp (Fig. 8). Furthermore,

the growth of these cells was also enhanced significantly by Pro-Hyp (Fig. 9). These studies suggest that ingested collagen peptide exhibits its effects on health and beauty through mechanisms unique to collagen peptide. On the other hand, about one third of the amino acids in collagen are glycine. Glycine is known to have a number of biological activities (Refs. 6, 7). Thus, collagen has unique properties that are not found in other proteins, and these properties may be the reason for the effects of collagen peptide ingestion on bones, joints, skin, hair and nails.



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Ingestion of collagen Peptide

Collagen report No.4

Collagen peptide is a popular supplement in Japan, and the market is currently growing rapidly because of increasing awareness of its effects on health and beauty. In this report, the amount of collagen ingested from diet is compared between Japanese adult males and females. This information will help develop novel products for both male and female consumers. Furthermore, up-to-date information on studies on the action mechanisms of ingested collagen peptide is summarized.

1) INGESTION COLLAGEN FROM DIET

comparison between japanese adult males and females

Collagen is a protein that is commonly present in animal-derived foods. Collagen molecules in food have a triple-helix structure and are assembled in collagen fibrils, which are further assembled in thicker collagen fibers. Collagen fibers play an important role in maintaining the structure and strength of body tissues, including skin and bones.

Like other proteins, collagen is a polymer of amino acids. However, the amino acid composition and sequence is markedly different from those of other proteins. Most proteins are composed of 20 types of amino acid; however, collagen contains an additional unique amino acid called hydroxyproline. During collagen synthesis, the amino acid proline is converted to hydroxyproline within the collagen structure.

The ratio of hydroxyproline in the collagen structure is called the “hydroxyproline factor”, and this is unique to each animal species. Therefore, one can calculate the amount of collagen in food products as follows: collagen (mg/g) = (hydroxyproline factor) × (hydroxyproline) (mg/g). The amount of hydroxyproline present can be determined by the dimethylaminobenzaldehyde method. Table 1 shows the amount of collagen present in some food products. One can calculate the amount of collagen in the daily diet using nutrition survey data obtained by a food recording method and the values in Table 1

Table 1 Collagen of food products. (Ref. 1)

Meat / Fish food products	Notes	Collagen content (mg/g)
Beef		7.5
Beef tendon		49.8
Pork		11.9
Pork chitterlings		30.8
Chicken thigh		15.6
Chicken wing tips		15.5
Chicken drumette		19.9
Chicken liver		8.6
Chicken gizzard		23.2
Chicken sternum cartilage		40
Ham		11.2
Tuna		5.7
Salmon	Skinless	8.2
	With Skin	24.1
Sierra	Skinless	10.4
	With Skin	12.8
Yellowtail	Skinless	9.7
	With Skin	16.2
Broiled eel		55.3
Daggertooth pike conger skin		76.6
Squid		13.8
Shrimp		11.5
Boiled and dried baby sardines		19.2
Sand eel		12.9
Short neck clam		11.00
Oyster		9.8

Table 2 shows the average protein intake data by Japanese according to the 2007 National Health and Nutrition Survey. A Japanese adult male takes in a total 77.7 g of protein per day, of which 42.1 g is animal-derived. These values are 64.9 g and 34.2 g, respectively, for a Japanese adult female

Table 3 Collagen intake by Japanese adult people

	Males	Females
Collagen (g)	77.7	64.9

The animal body comprises about 20% protein, and one third of the total protein is collagen. Thus, assuming that a Japanese adult male consumes the whole animal as part of his diet, he would take about 13 g of collagen (1/3 of 42.1 g). In a case of a Japanese adult female, she would take in about 11 g of collagen (1/3 of 34.2 g). Although humans are unable to consume some animal parts, such as large bones, it is likely that Japanese have been taking in considerable amounts of collagen, as most edible parts of fish and shellfish are major protein sources.

Table 3 shows the data obtained by Nippi and Kinjo Gakuin University on collagen intake of Japanese adults (Ref. 1). Japanese adult males take in 1.8 g of collagen in their daily diets, while Japanese adult females take in 1.7 g. Thus, there is little difference between males and females. However, these values are smaller than those in Table 2. At present, many Japanese prefer soft, lipid-rich meat instead of tougher collagen-rich sinew. Furthermore, it is more common now for people to ingest fish meat and leave the collagen-rich skin. Therefore, the amount of collagen ingested has decreased in Japan, as compared with other nutrients, and this may be why the effects of collagen peptide ingestion on health and beauty have become so apparent.

Table 2 Protein intake by Japanese adult people

	Males	Females
Total protein (g)	77.7	64.9
Animal-derived (g)	42.1	34.2

Many of the current consumers of collagen supplements in Japan are female. The data shown in Table 3 suggest that males could benefit from higher collagen peptide ingestion and could be promising consumers for collagen products.

Collagen-rich foods



Broiled eel



Salmon



Boiled & dried baby sardines



Beef tendon



Chicken gizzard

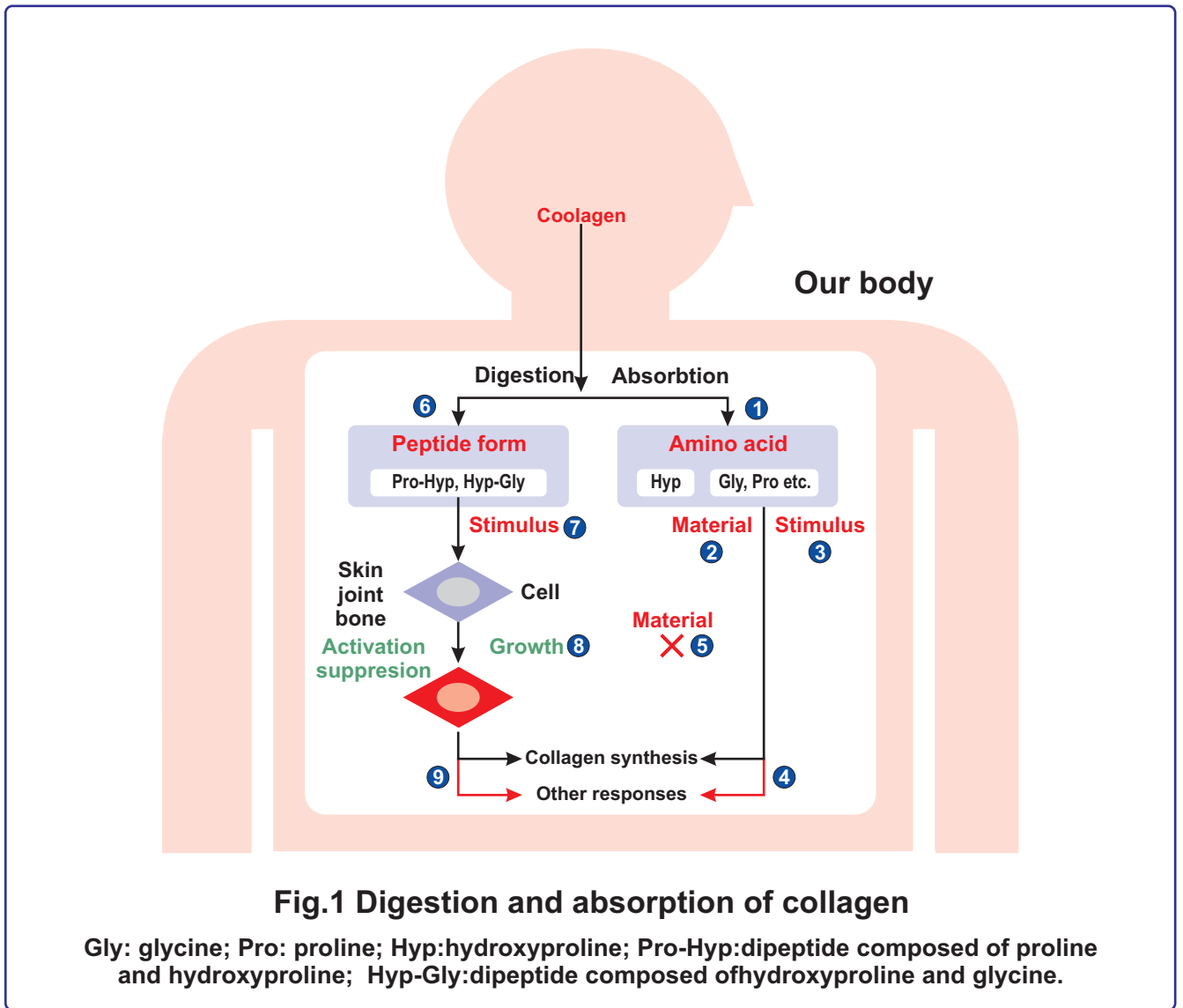


Chicken sternum cartilage

2) NUTRITIONAL ROLE OF COLLAGEN

Humans need to ingest sugars, lipids and proteins in order to maintain proper health. Vitamins and minerals are also necessary to regulate bodily functions, and dietary fiber is considered to be an important nutritional element for our health.

Ingested protein is digested to free amino acids and absorbed into the body. However, there is a considerable difference between collagen and other proteins in the manner of digestion and absorption. Figure 1 shows how collagen is digested and absorbed, and how the digested products work in the body.

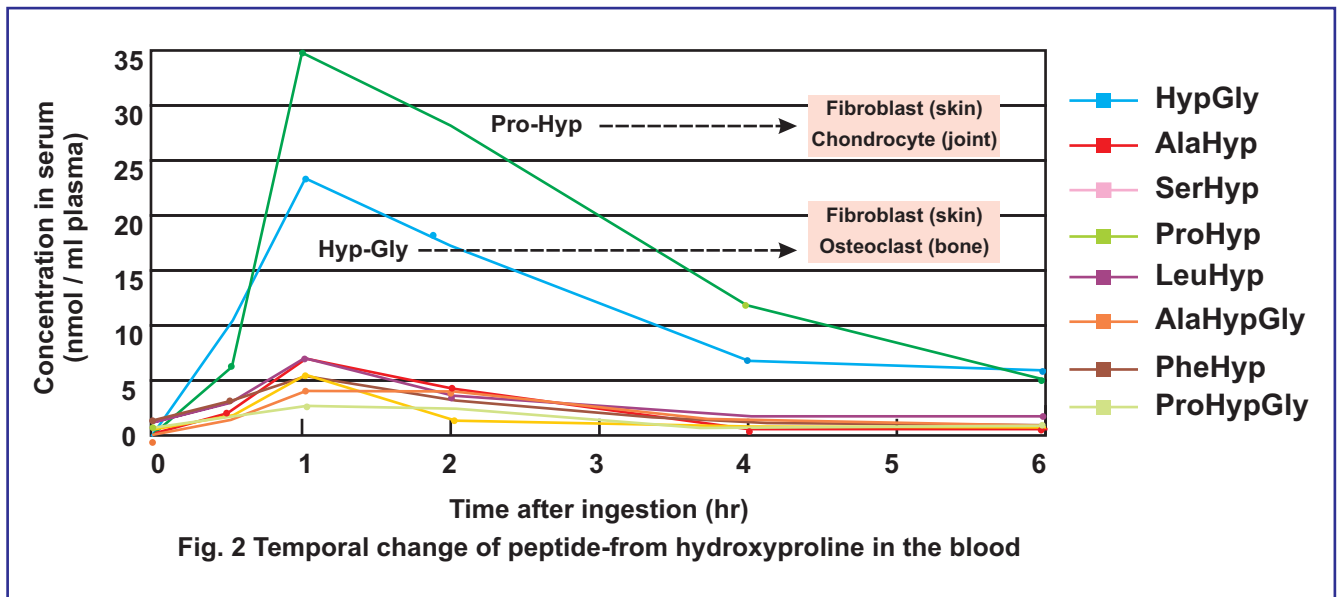


When collagen peptide is ingested, it is partially digested to amino acids and absorbed into our body (1). The amino acids can then be used as raw materials in collagen synthesis (2). On the other hand, amino acids can directly stimulate cells (3) and consequently affect collagen synthesis or other cellular responses (4). However, it should be noted that hydroxyproline is not incorporated into collagen (5) (Ref. 2), as hydroxyproline is the result of amino acid modification within the collagen molecule.

The unique aspects of the digestion and absorption of collagen peptide are shown on the left half of Fig. 1. When collagen peptide is ingested, hydroxyproline peptides such as ProHyp or Hyp-Gly appear in the blood (6). This indicates that collagen peptide is not completely digested to free amino acids, but is digested partly into small peptides, which can be detected in the blood (Refs. 3,4). These hydroxyproline peptides stimulate cells in the skin, joints and bones (7), and cell activation/suppression and/or growth is affected (8) leading to collagen synthesis or other cellular responses (9) (Refs. 4~6). The appearance of hydroxyproline peptides is due to the fact that digestive enzymes do not efficiently cleave the bond between proline/hydroxyproline and other amino acids, and that the ratio of proline/hydroxyproline is considerably higher (more than 10%) in collagen. Studies on the action mechanisms of ingested collagen peptide started in 2005 following the elucidation of the amino acid sequence of hydroxyproline peptides by Iwai et al. (Ref. 3). Further studies will help confirm the unique nutritional roles of collagen.

3) DIGESTION AND ABSORPTION OF NIPPI COLLAGEN PEPTIDE

Figure 2 shows the temporal changes in hydroxyproline peptides in the blood after ingestion of collagen peptide prepared by Nippi. Among the hydroxyproline peptides detected in the blood, Pro-Hyp and Hyp-Gly were the most abundant. Their concentrations increased at 1 h after ingestion and decreased thereafter. Pro-Hyp stimulates skin fibroblasts (Ref. 5) and joint chondrocytes (Ref. 6), and Hyp-Gly modulates functions of skin fibroblast (Ref. 6) and bone osteoclast (Ref. 7). These metabolic functions of Nippi collagen peptide indicate the importance of collagen peptide ingestion on health and beauty.



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