NIZONA
Japan

# Collagen

## Research Report


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# INDEX

# BENEFITS OF COLLAGEN

# EXECUTIVE SUMMARY

- 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.
- Ingestion of collagen helps healthy nail growth.
- The thickness of hair increased significantly after collagen ingestion for a continuous period.
- Research suggests that collagen brings similar results as whey protein when it comes to the development of muscle during strength training periods as a result. It is concluded that collagen has the same results as whey protein when it comes to muscle building effects.
- Collagen ingestion is beneficial in reducing the amount of triglyceride in blood.
- Collagen is used clinically to improve the amino acid imbalances in liver cirrhosis.
- Collagen fibrils in the Achilles tendon become thicker specifically in response to ingestion of collagen peptide.
- 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.

# 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.

"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.

Collagen Peptide is a high-quality bio peptide which is also known as "Super Collagen" because this is the form of collagen that our body can best absorb.

Fish derived collagen contains much larger amounts of the amino acids, glycine, proline and hydroxyproline than many other proteins. These amino acids are necessary for promoting healthy tissue growth by the cells themselves. Collagen embraces the benefits of "beauty from within".

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.

Comprehensive clinical studies have been carried out, highlighting the numerous positive effects of ingesting collagen.

## 2) EFFECTS ON SKIN

We carried out a double blind test in order to examine the effects of collagen intake on skin hydration. Young female volunteers consumed a drink containing 10 g of collagen or placebo every day for 2 months. Figure 1 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 μS) was larger than that of the placebo group (10.8 μS) after 1 month, and the difference became larger between the collagen group (203.5 μS) and the placebo group (113.7 μS) 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. Morgantie et al. also reported that skin hydration increased when a patient with dry skin ingested collagen.

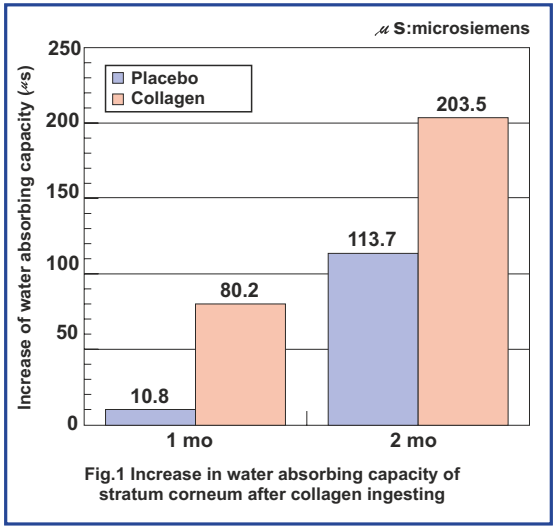



Fig.1 Increase in water absorbing capacity of stratum corneum after collagen ingesting

## 3) EFFECTS ON DERMIS

The dermis of the skin, 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).

Scientists had previously investigated the effects of ingestion of 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 and Table 1). These results suggest that ingestion of collagen peptide has beneficial effects that are not found with other types of protein lactalbumin (Fig. 2).**

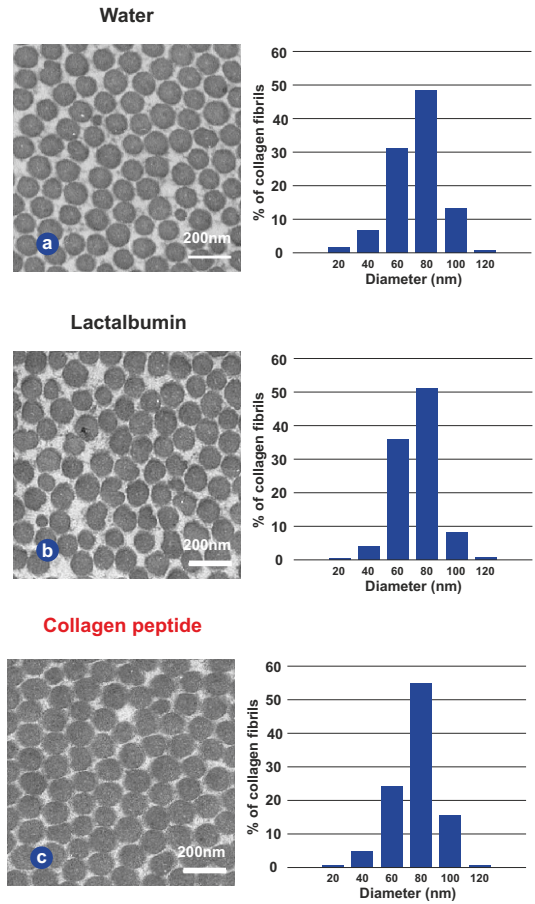



**Fig. 2 Effects of Collagen on dermis**

**Table 1. Effects of collagen peptide ingestion on collagen fibrils in the dermis (modified from Fig. 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 / $\mu m^2$) | 77.9 ± 2.7 | 74.3 ± 2.2 | 90.5 ± 1.8 * # |

**# Significantly different from the control (water) group and significantly different from the lactalbumin group**

## 4) EFFECTS ON NAIL AND HAIR

Nail defects are a common complaint among women. Rosenberg et al. showed that ingestion of collagen improved nail defects; they administered 7g 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 collage ingestion resulted in the reappearance of nail defects. Schwimmer et al. 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. 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

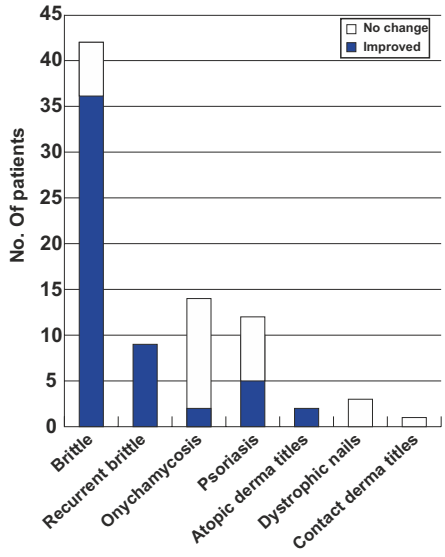



**Fig.3 Effects of Collagen ingestion on nail**

## 5) 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. When mice are given food containing 10% casein, bone mineral density becomes significantly lower when compared to mice given food containing 14%. Figure 4 shows bone mineral density of mice raised for 10 weeks with food containing 6% casein, 10% casein and 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 only 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.**

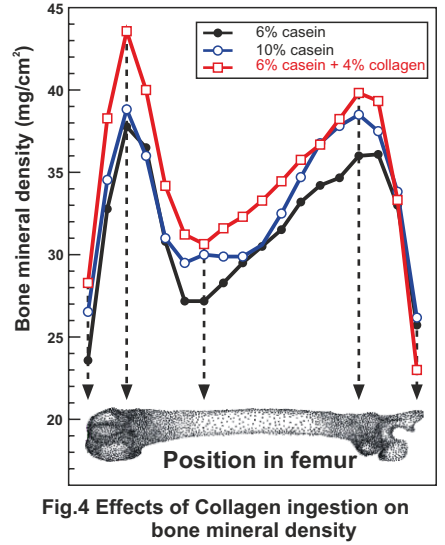



**Fig.4 Effects of Collagen ingestion on bone mineral density**

## 6) 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.

Scientific study on the effects of collagen peptide ingestion on the Achilles tendon has been performed - 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. 5a). The graph below 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 Fig. 5b. However, collagen fibrils were much thicker when rabbits ingested collagen peptide than when they ingested lactalbumin (Fig. 5c). **This suggests that collagen fibrils in the Achilles tendon become thicker specifically in response to ingestion of collagen peptide**

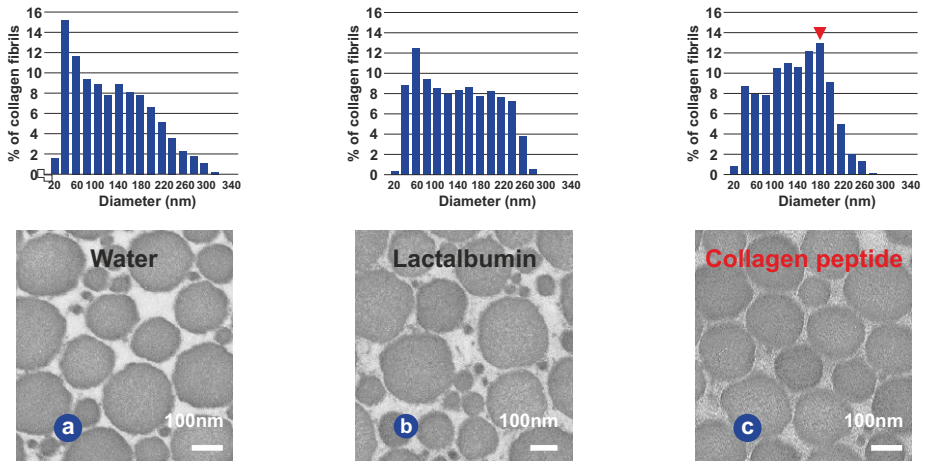



Fig. 5 Effects collagen peptide ingestion on collagen fibrils of the Achilles tendon

## 7) EFFECT ON MUSCLE

It has been reported that fish-derived collagen ("marine collagen") improves bone metabolism of an 'ovariectomized' rat caused by postmenopausal bone loss, resulting in osteoarthritis pain relief. We have also reported on the possibility of Marine collagen having bone strengthening effects, by increasing bone density using similar model. Further more, it was found that supplements containing collagen provided remarkable benefits in muscle building through a preliminary examination during a three month period.

Therefore, we experimented how the body tissues of American football college student players, whose sport requires physical strength, was affected by taking collagen supplements during the term of one year.

# FUNCTIONAL STUDY REPORT

**Title: Effects between the intake of Marine collagen and the building of muscle in college American football players.**

**Chart 1: The Status of each group regarding dietary fiber by FFQQg (Average nutrient intake of the two group)**

| | Marine Collagen Group (n=5) | | | Control Group (n=4) | | | |
|---|---|---|---|---|---|---|---|
| Energy | 33127 | ± | 85.7 | 3349.9 | ± | 164.9 | (Kcal) |
| Protein | 1024 | ± | 1.5 | 99.7 | ± | 2.7 | g |
| Fats | 98.0 | ± | 0.7 | 101.3 | ± | 4.8 | g |
| Carbohydrates | 484.2 | ± | 22.0 | 491.1 | ± | 27.0 | g |
| Calcium | 786.4 | ± | 38.7 | 748.4 | ± | 79.1 | mg |
| Iron | 10.24 | ± | 0.15 | 10.91 | ± | 1.23 | mg |
| Vitamin A | 662.52 | ± | 19.47 | 676.32 | ± | 64.92 | µg |
| Vitamin D | 8.76 | ± | 0.08 | 8.50 | ± | 0.15 | µg |
| Vitamin E | 9.01 | ± | 0.83 | 9.84 | ± | 0.58 | mg |
| Vitamin B1 | 1.54 | ± | 0.03 | 1.55 | ± | 0.15 | mg |
| Vitamin B2 | 1.66 | ± | 0.06 | 1.56 | ± | 0.13 | mg |
| Vitamin C | 81.35 | ± | 7.89 | 84.33 | ± | 13.23 | mg |
| **Total Amount of Dietary fiber** | 14.60 | ± | 0.93 | 16.30 | ± | 0.64 | g |

## 1) TEST METHOD

The target subjects were members of the Kantoh Collegeate American Football Association. This examination was approved by both research ethics committee (REC) and the individuals participating in the research.

We divided the players into two groups. One group (the "collagen group") took a powder mix of10g of a marine collagen supplement with 10g of whey protein after the club activities six times a week for a year. The other group (the "whey protein group") took 20g of whey protein without collagen. It is known that whey protein is high quality protein and has high content rate of essential amino acids.

Both groups had been having dietary nutrition surveys and body composition tests before the examination, after three months, six months and twelve months respectively. They also had medical checks through blood tests and urine tests after 6 months and 12 months.

## 2 ) TEST FINDING OF THE SURVEY

We found that both groups didn't have any problems about kidney function and liver function through the medical checks examined before the examination and after 6 months and 12 months. Moreover, nobody had any symptoms through the intake during the examination.

Their body weight, muscle weight and fat weight significantly increased during the initial three months (the 'muscle building' period) regardless of groups. This result suggests that marine collagen is a protein source same as whey protein.

However, the body weight, muscle weight and fat weight of the “whey protein group” decreased from the third month and reverted to their pre-examination condition after a 12 month period. This means, that the “whey protein group” could not maintain their body condition till the end of the testing period. On the other hand, members of the “collagen group” maintained their weight throughout the test period in a relatively consistent manner.

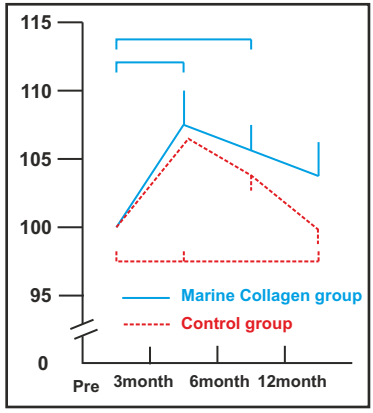
## 3) TEST CHARTS

**(Chart 1) Average nutrient intake of the two groups during the test period. There are no significant differences between the two groups.**

### 1. Graphic1 Body weight variations: Pretest, 3/6/12 months later

**a.** Both groups increased body weight during the initial three months.

**b.** The control group body weight returned back to the Pretest weight whereas the collagen group maintained a reasonable level of body weight approximately 5% over the pretest weight.

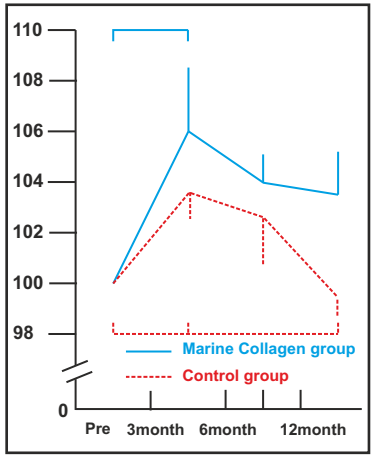



**Graphic 1: Rate of weight change from before the intake of Collagen (Body weight variations)**

### 2. Graphic 2 Muscle mass variations: Pretest, 3/6/12 months later.

**a.** The control group increased its muscle mass during the 'body building' period. However, after 12 months, their muscle mass returned to the same levels prior to the start of the test.

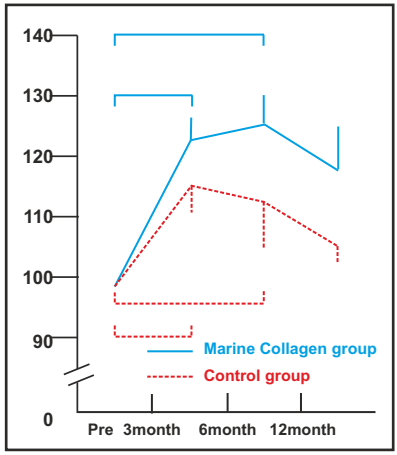
**b.** The collagen group also suffered some slight decrease in the muscle mass after the initial three months, this decrease was not significant.




**Graphic 2: Rate of muscle weight change from before the intake of Collagen (Muscle mass variations)**

## 3. Graphic 3 Body fat variations: Pretest, 3/6/12 months later.

**a.** The variations of body fat are similar to the results of muscle mass and body weight.

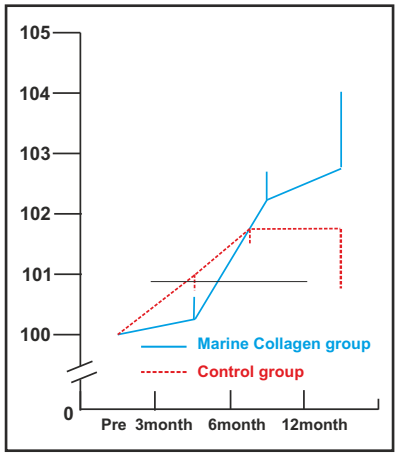



**Graphic 3: Rate of Fat amount change from before the intake of Collagen (Body Fat variations)**

## 4. Graphic 4 Systemic bone mass variations: Pretest, 3/6/12 month later.

**a.** Although not significant difference, during the one year test period both groups suffered increase in bone mass. There were no significant differences between the two groups.

**b.** The collagen group also suffered some slight decrease in the muscle mass after the initial three months, this decrease was not significant.




**Graphic 4: Rate of Bone mass change from before the intake of Collagen (Systemic bone mass variations)**

## These results prove that:

1. It is safe to consume collagen for long periods of time.
2. During the initial "body building" period of three months from the start of the test, both groups experienced increase in body weight, muscle and lipid mass. As a result, we conclude that collagen has the same results as whey protein when it comes to muscle building effects.
3. Additionally, with regards to the variation of body weight, muscle and lipid mass, the whey protein group returned to their pre-test condition in the period starting from after the "muscle building" period (3 months) to the end of the 12 months test. On the other hand, **the collagen group did not experience significant decrease of body weight, muscle and lipid mass even after the end of the season, when the strength training and test period ended.**
4. In a nutshell, the research suggests that collagen brings similar results as whey protein when it comes to the development of muscle during strength training periods.

## 8) EFFECTS ON TRIGLYCERIDE IN THE BLOOD

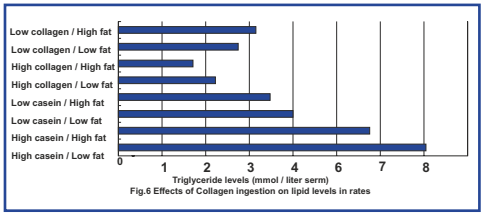


Fig.6 Effects of Collagen ingestion on lipid levels in rates

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. 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 6. It was found that triglyceride levels in blood decreased when rats were given collagen containing food than casein-containing food. Oliveira et al. and Wu et al. also reported the beneficial effects of collagen ingestion in reducing the amount of triglyceride in blood.

## 9) DIGESTION AND ABSORPTION OF COLLAGEN PEPTIDE

Figure 7 shows the temporal changes in hydroxyproline peptides in the blood after ingestion of collagen peptide . 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 and joint chondrocytes and Hyp-Gly modulates functions of skin fibroblast and bone osteoclast. These metabolic functions of collagen peptide indicate the importance of collagen peptide ingestion on health and beauty.

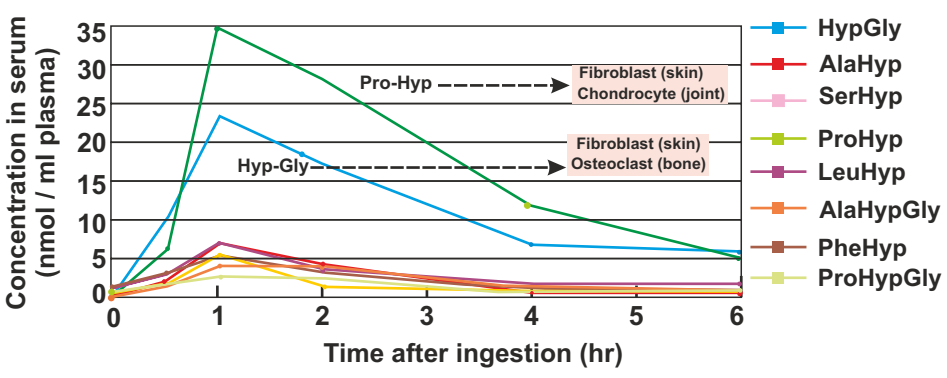


**Fig. 7 Temporal change of peptide-from hydroxyproline in the blood**

## 10) 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 8 shows how collagen is digested and absorbed and how the digested products work in the body.

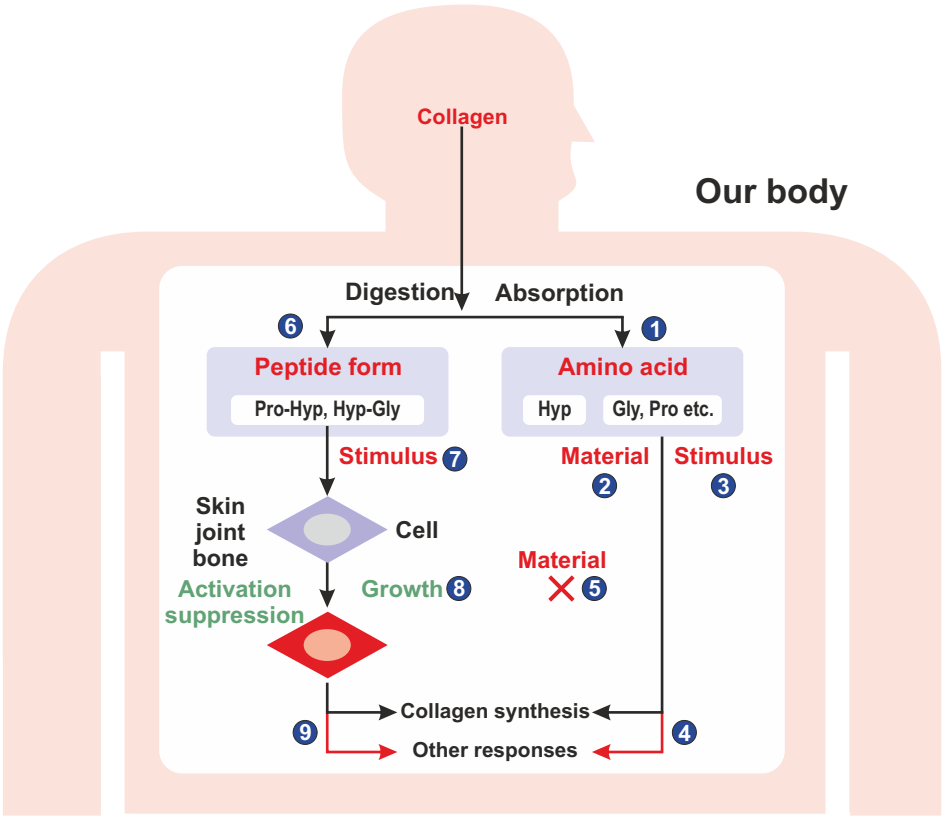



**Fig.8 Digestion and absorption of collagen**

Gly: glycine; Pro: proline; Hyp:hydroxyproline; Pro-Hyp:dipeptide composed of proline and hydroxyproline; Hyp-Gly:dipeptide composed of hydroxyproline and glycine.

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)** 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. 8. When collagen peptide is ingested, hydroxyproline peptides such as Pro-Hyp 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. 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)**. 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.

## 11) INGESTED COLLAGEN AS A SOURCE OF AMINO ACID

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.

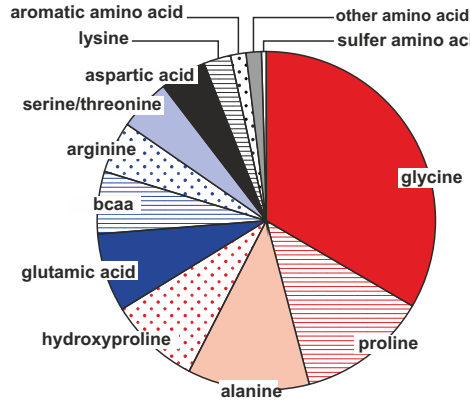



Fig.9 Amino acid composition of collagen peptide

Collagen is partly absorbed in peptide form. 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. In 2009, Shigemura et al. reported that the number of cells spreading from explanted mouse skin increased significantly after treatment with Pro-Hyp (Fig.10).

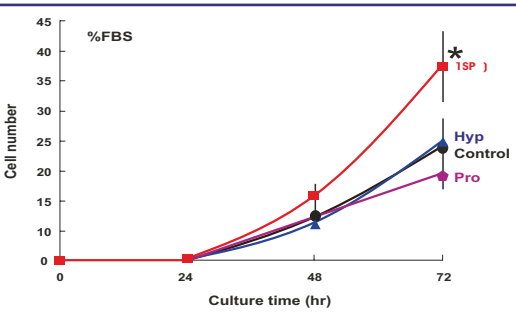



Fig. 10 Promotion of outgrowth cells from explanted mouse skin by Pro-Hyp * P<0.05

The 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. 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.

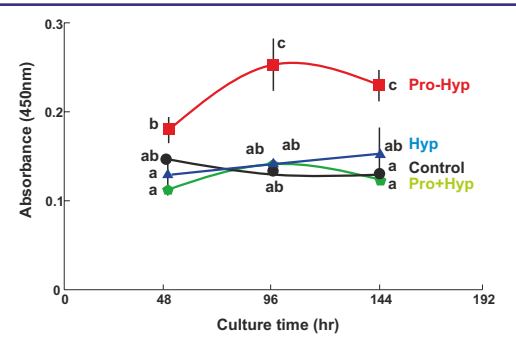



Fig. 11 Promotion of Cell growth by Pro-Hyp

## 12) MARINE COLLAGEN PEPTIDES PROTECT AGAINST EARLY ALCOHOLIC LIVER INJURY IN RATS

Marine collagen peptides (MCP) have been reported to exhibit antioxidative activity. The present study aimed to investigate the effects of MCP on early alcoholic liver injury in rats. Rats were administered with alcohol at a dose of 6g/kg body weight intragastrically per d to induce early injury, which was then evaluated by serum markers and histopathological examination. Treatment with MCP could reverse the increased level of serum aminotransferase and reduce hepatic hisological damage. In addition, MCP attenuated the alteration in serum superoxide dismutase and malondialdhyde levels. MCP also counteracted the increased levels of total cholesterol and TAG. However, no signicant difference was observed in the contents of alcohol dehydrogenase both in liver and serum protein of rats. These findings suggest that MCP have a protective effect on early alcoholic liver injury in rats by their antioxidative activity and improving lipid metabolism.
Being safe and multi-functional, MCP have a strong potential for long-term use as a supplement agent for kinds of illness involving oxidative stress, ALD, for example. However, further studies such as those with a longer-term design and human trials are needed.
In conclusion, the present study indicated that MCP could inhibit early alcoholic liver injury in rats based on the improvement of oxidative stress and lipid metabolism. Further studies are required to prove whether inammation and ADH are involved in the protective mechanisms.

## 13) 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.

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.

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 UVBirradiated 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.

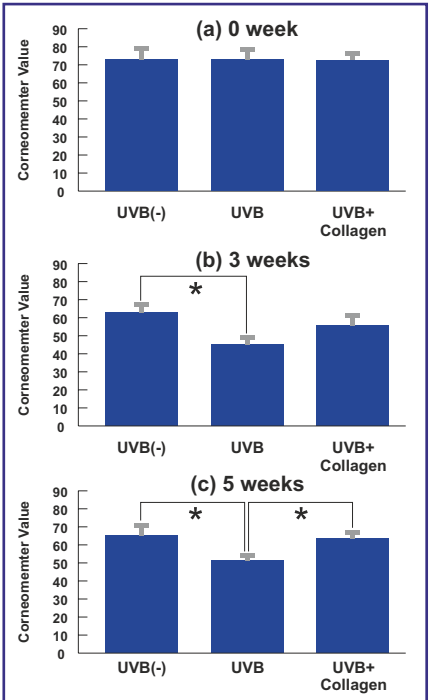



**Fig.12 Effects of UVB irradiations collagen peptide ingestion on water contents of stratum corneum**
UVB(-) : non irradiated group
UVB : UVB-irradiated group
UVB+collagen: UVB-irradiated and collagen-administered group
** p<0.05*

The dermis is also damaged by repeated UVB irradiation. 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 However, the amount of type I collagen did not decrease when collagen peptide was administered.

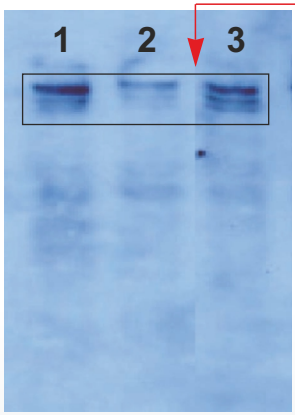



**Fig. 13 Effects of UVB irradiation and collagen peptide ingestion on amount of type I collagen in the dermis.**

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

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.

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