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Journal of Nutrition & Food Sciences
ISSN: 2155-9600

Maeda, J Nutr Food Sci 2018, 8:2
DOI: 10.4172/2155-9600.1000682

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Skin-Moisturizing Effect of Collagen Peptides Taking Orally

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Abstract

The skin is said to be "a mirror reflecting who you are inside". Indeed, beauty is not simply one's external appearance, but it also comes from within; beauty also comes from paying attention to both physical and mental health. Attention to have attractive skin has been on the rise in many age groups over the last decade; even women in their 20's often expresses the desire to look younger and fresher. Products containing collagen peptides drive the growth of the health and beauty and food and beverage industry. These products moisturize the skin, leading to their widespread popularity. Oral intake of collagen peptides has been found to increase the moisture content of the stratum corneum, thereby increasing the moisturizing capacity of the skin. Hydroxyproline (Hyp), produced as a result of digesting collagen peptides, increases the expression of serine palmitoyltransferase-2 and β-glucocerebrosidase, enzymes involved in ceramide synthesis in the stratum corneum. When taken orally, collagen peptides are absorbed by the body as Hyp or dipeptide, and this is believed to improve the moisturizing capacity of the skin by increasing the amount of ceramides in the stratum corneum. Consuming foods that beautify the skin is essential to maintain beautiful and healthy skin. Ingredients with skin-enhancing effects are absorbed by the body when ingested as food, and then distributed throughout the skin. This provides effective skin clarifying and anti-aging benefits. Substances that offer these actions are attracting the attention of not only consumers but also researchers interested in the science of beauty.

Keywords: Collagen peptides; Skin moisture; Skin viscoelasticity; Hydoxyproline; Prolylhydroxyproline; Ceramide

Introduction

"I want to keep my skin healthy and beautiful;" this is a common wish. Although the skin naturally weakens with age, various factors such as dry air during winter, ultraviolet ray exposure, especially during the summer, nutritional deficiencies, decreased resistance to oxidation, and other stress factors adversely affect the health of the skin. The skin is subjected to various influences that do not cause any subjective symptoms immediately but may result in small amounts of damage that accumulate over time [1-3]. Exposure to these factors occurs even more readily because of the societal stresses today. Some of these factors are inevitable, such as age-related changes. However, one can learn about the kind of lifestyle that is necessary to maintain attractive skin by gaining an understanding of the mechanisms by which the skin ages. Additionally, reviewing an individual's diet from the perspective of not only skin care, but also one's daily activities, can also help maintain healthy skin.

Results and Discussion

Preserving the beauty of the skin

Attention to the attractiveness of one's skin has been on the rise in many age groups over the last decade; even women who are in their 20s often express a desire to look younger. Beautiful skin refers to healthy and attractive bare skin. Although this concept refers to how the skin looks and feels, it also refers to the aspects of the skin that cannot be seen with the naked eye, such as its physiological health. The stratum corneum is the outermost layer of the epidermis and is in direct contact with the environment. This layer has an essential role of retaining moisture of the skin by producing a barrier that prevents moisture from evaporating and protects against physical and chemical stimulation.

The stratum corneum is composed of 15-20 layers of squamous endothelial cells, each one being approximately 1 μm thick (Figure 1). While the width of the stratum corneum is no more than 1/10 the width

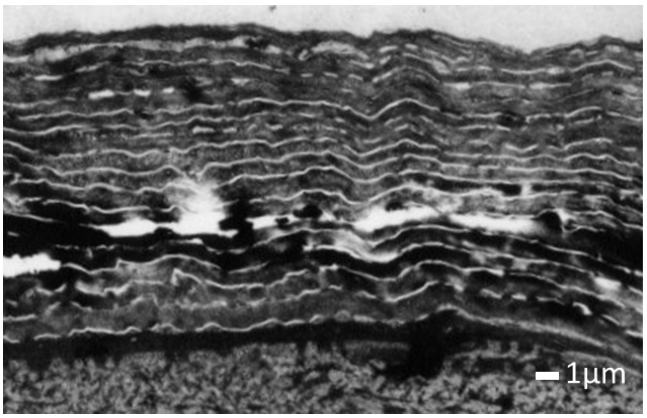

Figure 1: Electron microscopy of the stratum corneum. Epidermal cells are formed in the lower layer of the epidermis and the basal layer; these cells gradually rise to the outermost stratum corneum layer over a period of approximately 1 month. After reaching the stratum corneum, these cells are shed from the body after approximately 14 days, and this cycle is repeated. One of the roles of the epidermal cells is to maintain the endogenous environment, but it is the stratum corneum, which is made up of layers of dead epidermal cells, that actually completes this function. The stratum corneum is made up of 5-6 layers at the regions of the body where it is the thinnest, such as the vulva and the eyelids; it may contain 14-15 layers at regions such as the arms and torso, and may even contain 20 or more layers in areas such as the soles of the feet.

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Received February 19, 2018; **Accepted** March 20, 2018; **Published** March 31, 2018

Citation: Maeda K (2018) Skin-Moisturizing Effect of Collagen Peptides Taking Orally. J Nutr Food Sci 8: 682. doi: 10.4172/2155-9600.1000682

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of a single strand of hair, this layer plays a key role in the ability of the skin to retain moisture.

A healthy stratum corneum contains

(1) sufficient quantities of hydrophilic molecules,

(2) a favorable arrangement of ceramide and other intracellular lipids (lipids present in the extracellular space in the stratum corneum) between keratinocytes,

(3) a cornified cell envelope (CE),

(4) a well-maintained sebum barrier,

(5) properly functioning keratolytic enzymes, and

(6) an appropriate pH balance.

The ceramide and collagen peptides are intracellular lipids that play an important role in maintaining the moistness and fresh appearance of the skin. This paper will describe the effects of ceramide and collagen peptides to promote ceramide synthesis.

Intracellular lipids

Lipids extending into the intercellular space of the stratum corneum are called intracellular lipids, and in healthy skin, this layer is comprised of ceramide (37%), cholesterol (32%), long-chain fatty acids (16%), and cholesterol esters (15%); and they come together to form a multilayered lamellar structure [4-6]. Intracellular lipids act as a barrier between the water contained in the epidermis and the external environment and play a central role in preventing internal moisture from evaporating, while preventing external moisture from entering the body. If this barrier is damaged, excess moisture will escape through the stratum corneum, and further deterioration can lead to the development of allergic diseases of the skin, such as urticaria and atopic dermatitis [7,8].

Intracellular lipids are produced from precursor lipid molecules, but these precursor lipids are stored in the lamellar granules, the intracellular organelles found in the basal cells, spinous cells, and granular cells. The number of lamellar granules increases as epidermal keratinocytes differentiate and can comprise up to approximately one-third of the cytoplasm of granular cells. When granular cells differentiate into keratinocytes, the lamellar and granular membranes fuse, causing the release of the lipid molecules contained within the lamellar granules. It is here that the precursor lipid molecules undergo enzyme modification, and the resulting lipids extend into the extracellular space between corneocytes (Figure 2).

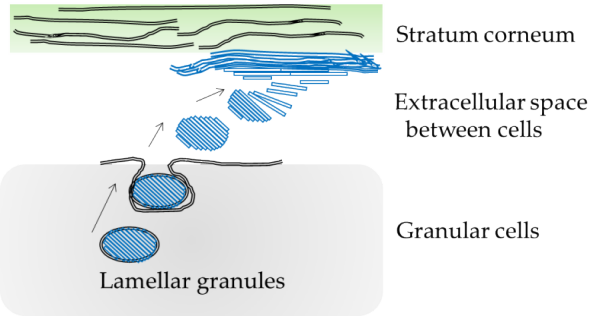

Figure 2: Formation of the lamellar layer. The keratinocytes and intracellular lipids formed during the process of keratinization make up the stratum corneum that is located at the outermost layer of the epidermis.

Intracellular lipids are arranged perpendicularly to the flattened keratinocytes to form a lamellar structure. This laterally packed array structure of the lamellar layer plays an important role in the barrier function of the skin. The diffraction pattern is primarily derived from the hexagonal (Hex) and orthorhombic (Ort) structures, and the Ort structural components are known to decrease largely in conjunction with the development of skin lesions, such as atopic dermatitis [9]. Lipids such as ceramides, cholesterol, and fatty acids are known to fill the intracellular space in this area. These intracellular lipids serve to form a framework comprised of numerous layers of water and fat, which functions to prevent the invasion of foreign substances. The intracellular lipids that make up the stratum corneum act as a barrier to prevent entry of external irritants, as well as providing protection from drying. There are 2 types of lamellar phases: the long periodicity phase (LPP), during which layers with a thickness of approximately 13 nm are stacked, and the short periodicity phase (SPP), during which layers of approximately 6 nm in thickness are stacked [9]. The moisture contained in the stratum corneum is kept constant and the skin's barrier function is maintained through preservation of the balance between these phases. "Acylceramide," which is an intracellular lipid with high hydrophobicity has the capabilities to suppress the evaporation of moisture from the stratum corneum, and it is known to be a key component in the formation of the LPP.

In theory, 12 ceramide isoforms are present in the human skin. These include 3 types of fatty acids: non-hydroxy fatty acids, α-hydroxy fatty acids, and esterified hydroxy fatty acids (ω hydroxy fatty acids), notated as [N], [A], and [EO], respectively [6]. Figure 3 displays the structure of ceramides found in the stratum corneum. In the (Figures 3 and 4) types of sphingoid molecules, dihydrosphingosine, sphingosine, phytosphingosine, and 6-hydroxy sphingosine, are shown and notated as [DS], [S], [P], and [H], respectively. Combinations of these molecules are theorized to give rise to 12 varieties of ceramides: CER[NDS], CER[NS], CER[NP], CER[NH], CER[ADS], CER[AS], CER[AP], CER[AH], CER[EODS], CER[EOS], CER[EOP], and CER[EOH] [10]. All these 12 types of ceramides, except ceramide 12, are present in the stratum corneum in the arms skin of humans. In a study comparing ceramide levels in healthy, adults and atopic and non-atopic dermatitis patients, it was found that the atopic dermatitis patients had insufficient levels of ceramide 1 [EOS] [10,11]. Although ceramide levels in these

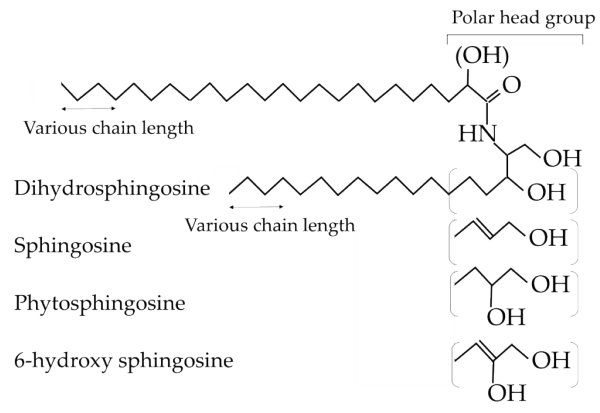

Figure 3: Structure of ceramide. Ceramides have a chemical structure in which fatty acids are acid-amine coupled to a sphingoid long chain amino alcohol (dihydrosphingosine, sphingosine, phytosphingosine, 6-hydroxysphingosine). Ceramide molecules form layers in the stratum corneum of the skin and gradually decrease with age; this thinning of the corneal ceramide layer is believed to be one of the causes of dry and sensitive skin.

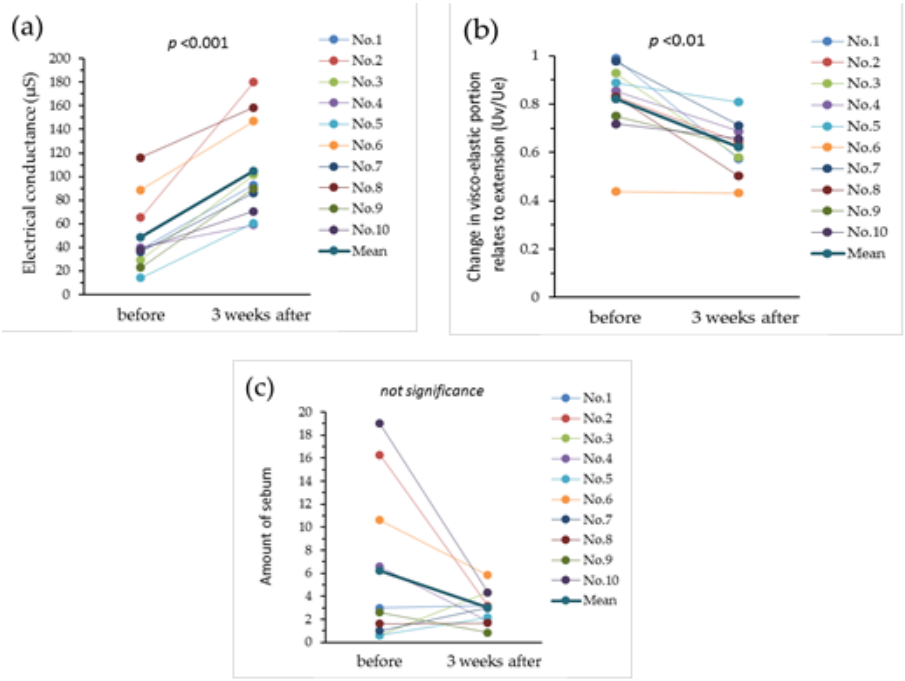

Figure 4: Improvement in cheek skin moisture content, viscoelasticity and the amount of sebum as a result of ingesting 10 g of collagen peptides. (a) skin moisture content, (b) viscoelasticity, (c) the amount of sebum. The corneal moisture content in the cheek skin of 10 adult female subjects (aged 33-61 years with the average age of 47.1 ± 8.6 years) was measured, using the SKICON-200 EX skin surface hygrometer (I.B.S. Co., Ltd., Shizuoka, Japan) before and 3 weeks after subjects cleaned their skin using a bar soap. They rested for 20 minutes in a controlled heat/humidity environment (temperature: 22.3°C ± 0.2°C, humidity: 31.9% ± 0.6%) and ingested 10 g of collagen peptides. Viscoelasticity and the amount of sebum of the subjects' cheek skin were also measured using the Cutometer MPA580 (Courage + Khazaka electronic GmbH Electronic GmbH, Köln, Germany) and Sebumeter (Courage + Khazaka electronic GmbH Electronic GmbH). A significant difference was confirmed in the levels of cheek stratum corneal moisture content and viscoelasticity between before and after collagen peptide ingestion. There was no significant difference in the sebum amount of the cheek between before and after collagen peptide ingestion. The SKICON-200EX is a device that measures moisture (in this case, stratum corneal moisture content) by determining electric conductivity (electrical conductance; unit: μS) using high frequency alternating currents. Electrical conductance is a measure of the ease by which electrical current flows when a current is discharged between metal electrodes in contact with the skin, utilizing the conductive properties of water present in the body. Basically, electrical conductivity (μS) increases proportionally with the increased moisture in the stratum corneal. The Cutometer MPA580 measures the skin viscoelasticity, using a suction method. Negative pressure is created in the device and the skin is drawn into the opening of the probe, then released quickly after a defined period of time. Change in visco-elastic portion relates to extension (Uv/Ue) obtained from the immediate extensibility (Ue) and the delayed extensibility (Uv) at the exposure to a constant negative pressure is used as an indicator of skin viscoelasticity. Succinctly, low skin viscoelasticity results in an elevated Uv/Ue, and Uv/Ue increases with age [29].

patients appeared to be deficient in comparison, they also had markedly reduced ceramide 3 levels and short chain fatty acid formation, as well as decreased ceramide 8 [NH] [12]. These decreases have been reported to be positively correlated with the extent of transepidermal water loss (TEWL) [12].

Of these molecules, the ceramide types present in the stratum corneum play an important role and are believed to be 30 or more times more prevalent than the ceramide types found in other organs. The stratum corneal ceramides are distinguished not only by the quantity in which they are found, but also by the diversity of their molecular structures [10,13]. Several types of ceramides are present in the stratum corneum; but, ceramides containing linoleic acid components either with or without ester bonds with acylceramide are the most common. Ceramide has a molecular structure in which fatty acids are acid-amine coupled to a sphingoid long chain amino alcohol group (dihydrosphingosine, sphingosine, phytosphingosine, 6-hydroxysphingosine). Ceramide molecules form layers in the stratum corneum of the skin that gradually thin with age, and reduced ceramide level is believed to be one of the causes of dry and/or sensitive skin [9].

Ceramide formation

The ceramide biosynthesis pathways active in the skin are divided roughly into 2 pathways: the *de novo* pathway and the salvage pathway. The *de novo* pathway is theorized to be critical to the production of ceramides within the stratum corneal intracellular lipids. The *de novo* ceramide synthesis pathway originates from 3-ketosfinganine, which is formed through the binding of sericin palmitate to L-serine by the action of serine palmitoyltransferase-2 (SPTLC2); it subsequently becomes sphinganine and then dihydroceramide via the action of various other enzymes and is finally stored as acylglucosylceramide, glucosylceramide, and sphingomyelin in lamellar granules contained in granular cells. The lipids stored in these lamellar granules are released into the extracellular space during the differentiation of granular cells into keratinocyte, after which glucose is liberated from acylglucosylceramide and glucosylceramide by β-glucocerebrosidase (β-GCase), leaving acylceramide and ceramide as respective reaction products [14]. Acylceramide is a molecular type specific to the epidermis and a ω-hydroxy fatty acid that is an acid-amine coupled to sphingol. The ω terminal of the molecule is primarily esterified with linoleic acid.

Acylceramide is an essential component of the epidermal barrier, and when acylceramide levels decrease, the skin becomes desiccated [15-17]. In addition, phospholylcholine is similarly released from sphingomyelin by sphingomyelinase 1 and sphingomyelinase 3,

resulting in the production of ceramide. Meanwhile, these ceramides undergo hydrolysis in the stratum corneum by ceramidase, an enzyme originating from the lamellar granules, to become sphingosine and fatty acids. Acylceramides are important for maintaining the barrier function of the skin and are produced from acylglucosylceramide by the action of β-GCase. Some ceramides are also produced from both glucosylceramide and sphingomyelin.

Elongation of fatty acids synthesized during ceramide synthesis occurs in the endoplasmic reticulum. After converting fatty acids to acytlCoA, the molecules are elongated by 2 carbon atoms through a single 4-step reaction cycle consisting of condensation, reduction, dehydration, and another reduction [18]. The first step is the rate-limiting step, catalyzed by a condensing enzyme (elongases). There are 7 condensing enzymes in mammals (ELOVL 1-7), each one exhibits a different substrate affinity. Epidermal keratinocytes produce an enzyme that extends the length of fatty acids (fatty acid elongase) and is capable of synthesizing long chain fatty acids (C22 or more) and very long chain fatty acids (C26 or more) in the skin. Among the condensing enzymes, ELOVL6 is required for C18 formation (C18:0 stearate), ELOVL3 is required for C18-C24 formation (C18:0 stearic acid, C20:0 arachidic acid, C20:1 gondoic acid, C22:0 behenic acid, C24:0 lignoceric acid). ELOVL1 is required for the formation of C20:0 or more fatty acids (C20:0 arachidic acid, C22:0 behenic acid, C22:1 erucic acid, C24:0 lignoceric acid, C24:1 nervonic acid, C26:0 cerotic acid, C26:1 hexacosenoic acid, C28:0 montanic acid). ELOVL1 plays a central role in C24 sphingolipid production. HeLa cells with decreased ELOVL1 exhibited decreased C24 sphingolipids and increased C16 sphingolipids. Mouse models of intentionally broken ELOVL1 died within 12 hours after birth [19]. The cause of death was dehydration [19]. The mice missing the gene coding for fatty acid elongase ELOVL1 exhibited skin barrier functional abnormalities, arising from decreased quantities of ceramides (including acylceramide), containing 26 or more carbon atoms in the epidermis, which resulted in stillbirth [19]. ELOVL1 converts long chain fatty acids to very long chain fatty acids. Acylceramide serves an essential function in the formation of the dermal barrier, and genetic mutations that affect its expression cause ichthyosis. Additionally, when an aqueous dye solution was placed on the skin in a typical location, the mouse turned into a deep blue color, and the stratum corneum barrier was virtually nonfunctional. Formation of long chain fatty acids does not occur in the stratum corneum, instead, it is believed to occur in the lamellar layer.

When comparing the differences in the amounts of ceramides containing C16 and C18 fatty acids [20] between a mouse with healthy skin and an atopic dermatitis model mouse, it was noted that C24, C26 very long chain fatty acids and extremely long chain fatty acids were abundant in the mouse with healthy skin, whereas C16 long chain fatty acid formation was significantly increased in the atopic dermatitis model skin [21]. This observation suggests that the reduction of extremely and very long chain ceramide synthesis can lead to issues with the skin. Several reports have stated that, both the moisture retention function and the barrier function of the skin decrease with the continuous application of ceramides containing C16 and C18 fatty acids [22]. Conversely, when ceramides with fatty acids longer than C20 are applied, both moisturizing and barrier functions of the stratum corneum improved [22]. A proportion of extremely long chain fatty acids of 50% or higher is indicative of a strong stratum corneum, while a proportion lower than 50% indicates a weakened stratum corneum that can lead to dry skin [21].

Collagen peptides as a food product to promote attractive skin

It is important to maintain the flexibility of the stratum corneum by increasing its ability to retain moisture and to keep the skin surface beautiful and clean. Using skin care cosmetic products with a moisturizing effect helps keep the surface of the skin fresh, but it goes without saying that proper dietary habits are also critical to maintaining beautiful skin internally. Although obtaining nutrients naturally from foods are ideal, if getting enough of these nutrients proves to be difficult, beauty foods with ingredients that improve the skin may be a solution. As such, the demand for such functional ingredients will be expected to continue to increase in the future.

It has been reported by several research institutes that among the various types of functional foods, collagen peptides that have been hydrolyzed to have lower molecular weights by using enzymes offer the most beneficial effects to beautify the skin. Collagen is a fibrous protein present in various tissues, such as the skin, bone, and blood vessels. Collagen comprises approximately 30% of the proteins constituting the human body. Forty percent of the human collagen resides in the skin, while 20% are in the bones and cartilage; it is also widely distributed throughout the body in locations, such as blood vessels and visceral organs. Collagen is also known to serve as the primary component of the dermal matrix. The deterioration of this matrix with age is considered to be one of the several factors giving rise to wrinkles and sagging skin.

The primary structure of the collagen protein differs substantially from that of other proteins in the following manner:

(1) approximately 35% of the collagen protein is made up of the simplest amino acid, glycine (Gly),

(2) a special amino acid known as hydroxyproline (Hyp) comprises approximately 10%, and

(3) proline (Pro) makes up another 12 percent. Examples of collagen-rich foods include broiled eel, conger eel skins, beef sinew, and chicken skin.

To discover the absorbability of collagen peptides, their migration into the blood stream by instructing subjects to ingest collagen peptides at different molecular weights, derived from fish scales (average molecular weights: 5,000 and 1,300), was found that lower molecular weight results in greater absorption [23]. In addition, by lowering the molecular weight of collagen proteins, its gelling ability disappears; therefore, it becomes more readily dissolved in water, which is advantageous from the viewpoint of commercialization as a beverage product. Collagen peptides with low molecular weight are incorporated into foods that promote beauty because of their smooth digestibility and absorption. Pork skin collagen peptide and fish scale collagen peptides are believed to exhibit the same absorbability if they have the same average molecular weight. An *in vivo* kinetics test was conducted to investigate the transdermal movement of ^{14}C-labeled collagen hydrolysate in mice; after oral ingestion, the labeled amino acid was detectable in the skin of the mice, and it peaked after 12 hours [24]. Another study measured the quantity of Hyp in the skin of mice after gelatin or collagen peptide ingestion. The quantity of Hyp detected in the skin was not significantly different from those detected in mice belonging to the control group that received thermally denatured collagen only; however, high quantities of Hyp were also detected in the skin soluble fraction [25].

Efficacy of collagen peptides for promoting attractive skin

The positive effects of taking collagen peptides orally on the skin have been tested by several independent research institutes. It has been demonstrated to increase stratum corneal moisture content, which is an indicator of the health of this skin layer. These tests have also demonstrated that collagen peptide consumption can improve skin viscoelasticity.

1) The results of a double-blind study on the effects of consuming food that contained collagen peptides showed that the skin viscoelasticity has improved in 44 healthy adult women after 8 weeks of continuous use. In addition, improvements in the subjective conditions regarding makeup or cosmetic maintenance, skin smoothness, and pores were confirmed, using a visual analog scale [26].

2) In a double-blind study, 214 women aged 25-45 years were divided into 5 groups and instructed to consume 2.5 g, 5 g, or 10 g of collagen peptides from fish scale, daily for 4 weeks. It was observed that moisture content of the stratum corneum increased in both the placebo group and the exposure groups. However, when researchers compared the degree of change between the groups, the most significant differences were observed in the groups that consumed 5 g and 10 g of collagen peptides based on the Williams test [27].

3) In a study of 69 adult female subjects aged 35-55 years who were given 2.5 g or 5 g of collagen peptides daily for 8 weeks, skin viscoelasticity at weeks 4 and 8 was found to be improved compared to that of the placebo group. In addition, improvement in skin viscoelasticity and stratum corneum moisture content were observed as a result of a stratified analysis of subjects aged 50 years or older [28].

4) In a study of 10 adult female subjects aged 33-61 years who ingested 10 g of collagen peptides once daily for 3 weeks, all of them exhibited improved cheek skin stratum corneum moisture content and viscoelasticity (Figure 4).

In addition, consumption of collagen peptides has been reported to improve strength of nails [30-33], increase blood flow to the fingers [34], thicken thinning hair [35,36], in addition to other beneficial effects.

Metabolism of consuming collagen peptides orally

When collagen peptides are consumed as food, the majority is broken down into component amino acids in the digestive system and absorbed. Dipeptides such as Hyp and tripeptides such as Pro-Hyp, Ala-Hyp, Ala-Hyp-Gly, Pro-Hyp-Gly, Leu-Hyp, Ile-Hyp, Phe-Hyp, Ser-Hyp-Gly, and Gly-Pro-Hyp [37] also enter the blood stream and can be absorbed as dipeptides or tripeptides. In general, dipeptides and tripeptides are rapidly broken down in the blood, but prolylhydroxyproline (Pro-Hyp) is not rapidly metabolized, and its blood concentrations of several tens of μM can persist even after 4 hours [38]. Pro-Hyp has also been shown to be capable of reaching the joint and skin tissues after ingestion [39]. Based on these findings, the primary skin metabolic products of collagen peptides taken orally include amino acids, such as Hyp, Pro, and Gly; dipeptides, such as Pro-Hyp, as well as various tripeptides. Each of these compounds is thought to have some positive effects on the skin.

Impact of collagen peptides, Hyp, Pro, Pro-Hyp, and Gly on ceramide synthesis

To investigate whether ceramide biosynthesis is involved in the skin improving effect of collagen peptides, I examined the effect of collagen peptide metabolites on the SPTLC2 and β-GCase enzymes involved in ceramide biosynthesis. I also examined the effects of collagen peptides, Pro, Hyp, Pro-Hyp, and Gly on SPTLC2 and β-GCase mRNA activity, using human 3-dimensional cultured skin models; the quantity of SPTLC2 mRNA increased by the collagen peptides and Hyp (Figure 5). It was found that the quantity of β-GCase mRNA also increased by the collagen peptides, Hyp, and Pro-Hyp (Figure 5). Figure 6 displays a fluorescent microscope image of β-GCase activity in preparing tissue sections after collagen peptides and Hyp were allowed to react in a human three-dimensional cultured skin model. When compared with the control group, it was observed that β-GCase activity between the granule layer and the stratum corneum has increased based on collagen peptides and Hyp levels. In contrast, this effect was not observed with respect to Pro and Gly. As Hyp is hydrophilic, a mechanism in which increased stratum corneal Hyp concentration results in greater moisture in the stratum corneum is conceivable. In addition, because other collagen peptide metabolites have not yet been investigated, the involvement of oligopeptides derived from collagen peptides during ceramide biosynthesis cannot be ruled out. Because the quantity of

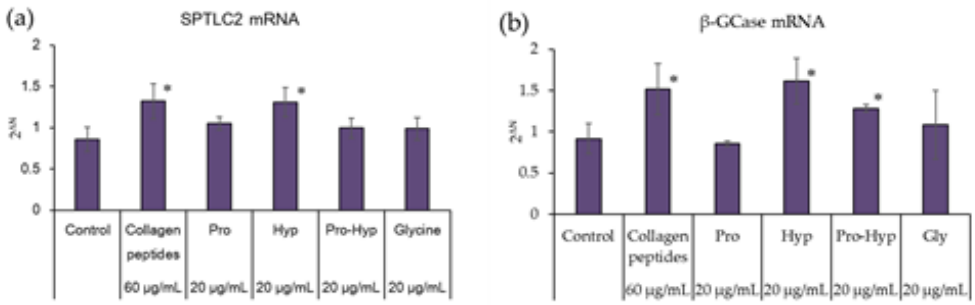

Figure 5: The effects of collagen peptides, Hyp, Pro, Pro-Hyp, and Gly, on levels of SPTLC2 and β-GCase mRNA in cultured human epidermis models. (a) quantity of SPTLC2 mRNA, (b) quantity of β-GCase mRNA. n=3, mean ± SD, * $p < 0.05$ vs. control. Cultured human epidermis models were prepared over a 4-day culture period while being exposed to 60 μg/mL collagen peptides, 20 μg/mL Pro, 20 μg/mL Hyp, 20 μg/mL Pro-Hyp, and 20 μg/mL Gly in 0.5 mL of a specialized culture medium. RNA was extracted, using an RNA extraction kit (RNeasy Protect Mini Kit, Qiagen K.K., Tokyo, Japan). Levels of SPTLC2 and β-GCase mRNA expression were measured using real-time PCR (ABI PRISM 7900HT, Applied Biosystems Co., Ltd., Forster City, CA, USA), performed with the One Step SYBR® Prime Script™ RT-PCR Kit II (Takara Bio Inc., Shiga, Japan). The primers used were purchased from Qiagen. The number of specimens used for each test was n=3. During analysis, we determined the threshold cycle Ct, as well as the difference between the housekeeping gene (G3PDH) and the Ct value of the target gene (ΔCt: [Target gene Ct]- [Housekeeping gene Ct]); the difference between this value and the test specimen (ΔΔCt) was used to determine the rate of gene expression.

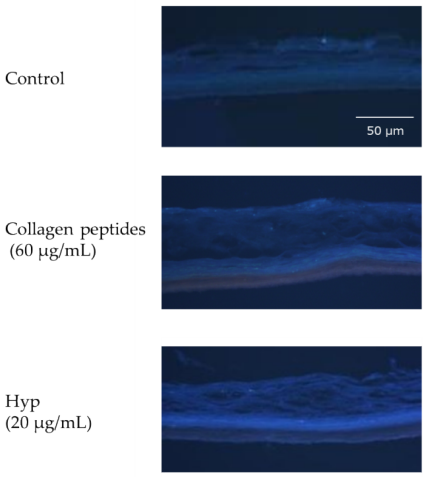

Figure 6: The effects of collagen peptides and Hyp on the action of β-GCase in cultured human epidermis models. Human epidermis models cultured with and without exposure to collagen peptides or Hyp (20 µg/mL) were frozen in liquid nitrogen, after which a tissue cross section was prepared using a cryomicrotome and then fixed for 5 minutes at 4°C in 4% paraformaldehyde/phosphate buffer (PFA). After rinsing with PBS, 100 µL of a 20 mM phosphate buffer solution (pH 5.6) containing 0.5 mM of 4-methylumbelliferyl-β-D-glucopyranoside was added and reacted at 37°C for 1 hour. A glass lid was placed over the solution, which was then observed using a fluorescent microscope (Olympus Corp., Tokyo, Japan). (a) β-GCase activity in the control human epidermis model; (b) β-GCase activity in the human epidermis models cultured under exposure to collagen peptides (20 µg/mL), (c) β-GCase activity in human epidermis models cultured under exposure to Hyp (20 µg/mL).

Hyp in the blood decreases with age [40], foods containing collagen peptides are believed to have anti-aging effects.

Effects of Gly

Thirty-five percent of the collagen protein are comprised of Gly, so the physiological effects of Gly cannot be ignored when considering skin beautifying effect of collagen peptide consumption. Gly is the smallest amino acid and is one of the nonessential amino acids. Additionally, Gly has been reported to improve "sleep quality" [41,42]. When Gly is consumed before sleep, the body is believed to more readily enter a state of deep natural sleep. Furthermore, consumption of Gly has been found to decrease feelings of tiredness and improve overall mood the following morning.

Sleep deprivation is believed to be an enemy of beauty, and getting enough sleep is essential to maintain healthy and beautiful skin. Skin cell division and regeneration are promoted by growth hormone secretion. Secretion of growth hormones is influenced by the body clock (circadian rhythm), and greater secretion occurs during the non-REM sleep that occurs immediately after falling asleep. For example, if a person goes to sleep at around 11 p.m., the period during which non-REM sleep is most likely to occur is between midnight and 1 a.m. Growth hormone promotes cell division and stimulates skin regeneration. Without sleep, growth hormones will not be secreted, and skin regeneration will not take place. If one continues to follow irregular sleeping patterns, his or her circadian rhythm will be disrupted, resulting in diminished immune function, hence autonomic nervous system disorders, which can lead to a variety of issues with the skin. This can also cause increased male hormone secretion, which can result in acne, arising from excessive sebum production as well as skin deterioration due to disrupted cell turnover.

Inner beauty

Proper dietary habits and nutritional supplements, adequate exercise and sleep, and the living environment are believed to play important roles in having a healthy skin. In some cases, it may be necessary to supplement individuals' diet with nutritional supplements if certain nutrients are lacking, but it is important to ensure that the correct amounts are taken. Taking more than the recommended dose will not increase the effectiveness of various vitamins and minerals, because health benefits from the replenishment of essential nutrients over a long period of time. Diets containing excessive amounts of junk foods or those that do not provide a proper balance of nutrients will not deliver the necessary nutrients to the skin and hair, leading to skin damage and split hair. In addition, hormone balance in women can be disrupted and menstruation may stop. If amenorrhea continues for more than a few months, female hormones in the body may decrease to levels typical of the post-menopausal period, even in younger women. Because female hormones are involved in the production of hyaluronic acid and collagen, deficiencies in these hormones can adversely affect the viscoelasticity of the skin and hair, as well as bone density.

In addition, using quality cosmetics and using them correctly are essential for skin health. To maintain the attractiveness of the skin, daily moisturizing is important; although cosmetic products are designed mainly to cleanse, moisturize, and beautify the skin, these products also attempt to work on the dead skin cells in the outermost layer of the skin, the stratum corneum; however, they do not improve the structure and/or function of the skin as defined by the Pharmaceutical Affairs Law. Furthermore, because the skin does not have digestive function similar to that of the stomach, which is capable of regulating the entry of ingredients into the body, the use of cosmetics that contain proteins, peptides, or potentially allergenic ingredients on areas of rough or damaged skin (e.g. rashes) may present the risk of developing allergic reactions. Therefore, good-quality cosmetic products must be very safe and should be composed of ingredients that do not readily penetrate the skin and are formulated on the premise of being used on healthy skin. Thus, to improve the conditions of the skin, individuals should take advantage of not only cosmetics, but the benefits of nutritious foods. People must strive for maintaining healthy diets and a lifestyle to promote a healthy body and mind, in addition to external beauty. Success in each of these areas will promote an individual's "inner beauty."

A proper diet is indispensable to maintain beautiful and healthy skin, because nutrients such as proteins, lipids, carbohydrates, vitamins, and minerals that support the structure and functions of the skin can often be acquired from foods only. A good diet is fundamental to improving skin disorders that cannot be resolved with cosmetic products. Consumption of foods that can positively affect the skin, such as collagen peptides [43], hyaluronan [44], N-acetylglucosamine [45], rice-derived glucosylceramide [46], royal jelly [47], and miso [48], are essential for maintaining the internal and/or external beauty of the skin.

Conclusion

The ceramide and collagen peptides are intracellular lipids that play an important role in maintaining the moistness and fresh appearance of the skin. This paper describes the effects of ceramide and collagen peptides to promote ceramide synthesis. Collagen peptides that have been hydrolyzed to have lower molecular weights by using enzymes offer the most beneficial effects to beautify the skin. The positive effects

of taking collagen peptides orally on the skin have been tested by several independent research institutes. Oral intake of collagen peptides has been proven to increase the moisture content of the stratum corneum, thereby increasing the moisturizing capacity of the skin. The primary skin metabolic products of collagen peptides taken orally include amino acids, such as Hyp; dipeptides, such as Pro-Hyp, as well as various tripeptides. Hyp increases the expression of SPTLC2 and β-GCase, and Pro-Hyp increases the expression of β-GCase in cultured human epidermal model. Hyp and Pro-Hyp are considered as involved in the induction of ceramide synthesis in the stratum corneum by oral intake of collagen peptides. When taken orally, collagen peptides are absorbed by the body as Hyp or dipeptide, and this is believed to improve the moisturizing capacity of the skin by increasing the amount of ceramides in the stratum corneum.

Acknowledgments

I thank Ms. R. Yamasaki, Ms. N. Ohshio, Ms. N. Iwashita, Ms. N. Okita, Ms. R. Yamaguchi, Mr. T. Takahashi, Mr. M. Shimotori, and Mr. M. Fukuzawa which were the student of School of Bioscience and Biotechnology of Tokyo University of Technology for technical assistance.

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