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Report

The effect of ingestion of an ethyl α-D-glucoside, a fermented product, on human skin

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Measurements were taken at 30 places on the inner side of both upper arms each week as part of various drinking tests, using a device that quantifies the measurements as the collagen score. In the first experiment, an immediate increase in the collagen score by 8 points in the first week and a continuous increase of 20 points in the fourth week were observed. In the second experiment, immediate and continuous effects were observed, with increase of 6 and 10 points in the collagen score in the first and fourth weeks, respectively. The third experiment showed that the collagen score in the first and second weeks increased by 4 and 9 points, respectively, and the keratinous moisture content increased by 3 and 8 points, respectively. These findings indicate that ethyl α-D-glucoside intake improves both the resilience and glow of the skin.

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INTRODUCTION

Ethyl α-D-glucoside (α-EG), which is the glycoside of the third ingredient in sake after ethanol and glucose, was first discovered in human urine in 1971.[1)] α-EG is synthesized from ethanol and maltose or longer oligosaccharides, which are produced

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through multiple parallel fermentation of sake from rice.[2] α-EG content in sake is the highest in pure rice wine (*Junmaishu*), which is 0.6 to 0.8%. It has been reported that the addition of α-glucosidase at the start of the conventional sake manufacturing process, increases α-EG concentration to 3% or higher.[3] α-EG is a sweetener with approximately 70% of the sweetness of glucose and slow-acting bitterness. According to the Liquor Tax Law, which limits the quantity of raw material, the maximum quantity of α-EG that does not cause bitterness is 2%. For this reason, it is necessary to maintain high α-amylase and α-glucosidase activities in *koji*, glucoamylase activity is limited by the minimum amount of glucose supply for alcohol fermentation. The enzyme activity balance between α-glucosidase and glucoamylase produced from *koji* is also important for α-EG production.

Metabolic research has shown that α-EG is adsorbed into the bloodstream through glucose transporters in rat intestines and is excreted via urine within several days.[4] It was also found that 80% of the amount consumed by humans is excreted within 24 h. α-EG leads to increased proliferation of human epidermal keratinocytes, and its application to skin roughened by UV radiation led to a decrease in transdermal moisture evaporation in a mouse model.[5] Oral intake of α-EG provided keratinous balance to the keratinocytes and improved roughened skin in a similar animal experiment.[6] α-EG application led to improvement in roughened skin through oral intake in animal experiments, suggesting that α-EG can be transported to the whole body through the bloodstream, and it can pass to epidermal keratinocytes through skin capillaries, resulting in the maintenance of their keratinous balance. Furthermore, the addition of α-EG at a very low concentration of 0.48 μM leads to activation of human adult skin fibroblast cells and production or secretion of collagen I.[7] These finding from previous studies indicate that α-EG could direct affect the fibroblasts in the dermal layer of skin in humans. Furthermore the transcript levels of type I collagen genes, *COL1A1* and *COL1A2*, were reported to be increased by 152 and 130%, respectively, and those of the type III collagen gene, *COL3A1* by 132% at an α-EG concentration of 0.48 μM.[7] While the above-mentioned cell experiments and gene expression experiments were

performed using pure α-EG, it was reported that the consumption of fermented sake or fermented shochu, containing 0.48 μM α-EG, leads to a significant increase in collagen levels in fibroblast cells.

Based on these reports and observations, we investigated the effect of α-EG in sake by oral intake. We used the collagen score in the dermal layer to evaluate the effect of sake consumption. In this study, we demonstrate the characteristic increase in collagen scores within one week in response to sake consumption and dose dependence of significance on the amount of α-EG in sake.

Materials and Methods

Study design

This study was conducted from January to December 2017, in compliance with the principles of the Declaration of Helsinki; written informed consent was obtained from all participants before the initiation of the study. All experiments were approved by the ethics committee of the Kanazawa Institute of Technology on October 12, 2016 (No. 0156).

Preparation of amazake

Amazake was prepared by mixing 100 g of sake lees (0.6 %w/w α-EG, 9.7 %v/w ethanol) and 200 mL of water while heating until the lees dissolved. This was used as the third test sample.

Measurement of collagen score

Dermalab® skin analysis system (Cortex Technology Aps, Hadsund, Denmark) was used to measure collagen scores. In principle, the ultrasonic echo shows collagen density in the dermal layer to be white, yellow, and pale green from high to low on an image and quantifies the density as a collagen score. Using this device, we performed ultrasonic diagnostic imaging of the dermal layer of the skin, as indicated in Fig. 1, each week, before and after each experiment. All experiments were performed at a temperature of 20-25℃ and humidity of 50-60%. In the first experiment (January-February 2017) panellists were 21-22 year-old individuals (five males and three females) who do not usually drink 180 mL of sake containing 1.7% α-EG (17 %v/v ethanol) per day (3.1 g/day of α-EG) in an ordinary drinking manner, during regular meals, and before sleeping. After eight days, the collagen score was measured at five points for each of the five sections of the inner side of both arms, as indicated in Fig. 1. The collagen score was measured before the start of the

experiment and at the end of each week during the 4-week experiment. The scores after excluding the maximum and minimum values from the 50 sites were analyzed (15 places×2 = 30 places). The second experiment (February-March 2017) was performed by five 23-48 year-old female panelists who do not usually drink sake. They took a normal diet and 50 mL of sake containing 1.1% α-EG (16 %v/v ethanol) for 6 days during dinner (0.55 g/day of α-EG). Collagen scores were measured before the start of the experiment and after the end of each week for 6 weeks. The third experiment (December 2017) was performed by four female 40-59 year-old female panelists, who consumed 100 g of sake lees, containing 0.6% α-EG, per day (0.6 g/day of α-EG content) in the form of *amazake* for 7 days as part of their normal diet. The collagen score and keratinous moisture content were measured before the intake and at the end of each week for 2 weeks. The fourth experiment (October-November 2017) was performed by eight 24-63 year-old female panelists, who did not usually drink sake. They consumed a normal diet and 40 mL of sake, containing 0.8% α-EG (16 %v/v ethanol), for 14 days during dinner (0.32 g/day of α-EG). Collagen scores were measured before the start of the experiment and after the end of each week during the 4 weeks experiment.

Measurement of keratinous moisture content

The keratinous moisture content in the human dermal layer was measured using a Corneometer® CM 825 device (Courage + Khazaka Electronic GmbH, Cologne, Germany). The third experiment was performed by four female 40-59 year-old female panellists, who took 100 g of sake lees containing 0.6% α-EG per day (0.6 g/day of α-EG content) in the form of *amazake* for 7 days as part of their normal diet. Keratinous moisture content was measured before and at the end of each week in the 2-week experiment.

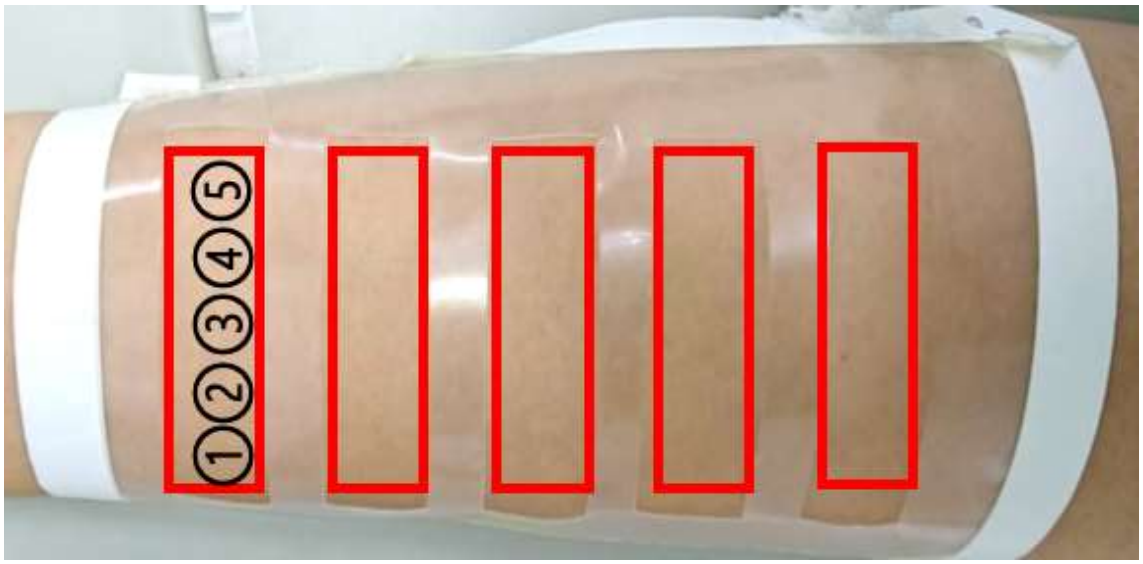

Fig. 1. Test area in the inner side of a forearm.

Measurements were performed at room temperature (20-25℃) and humidity (50-60%). We measured the collagen score and the keratinous moisture content by dividing the inner side of both arms into five sections (15 places×2 = 30 places) .

Results

First experiment

In the first drinking test of sake containing 1.7% α-EG, conducted by 21-22 year-old panelists, there was an increase 8 points in the collagen score the first week of sake consumption and 20 points during the fourth week compared to that before sake consumption. An immediate effect of collagen secretion was also observed in an experiment at a cellular level. In accordance with the increase in the collagen scores mentioned above, the ultrasonic echo image showed a significant increase in white dots in the dermis layer (most evident in the deepest region of the dermis). Although the increase in the collagen score was the highest after the first week of sake consumption, the increase was observed even after the termination of sake intake at the end of the first week. The results indicated continuous stimulation of fibroblasts for at least two weeks without sake intake(Fig. 2A, 2B).

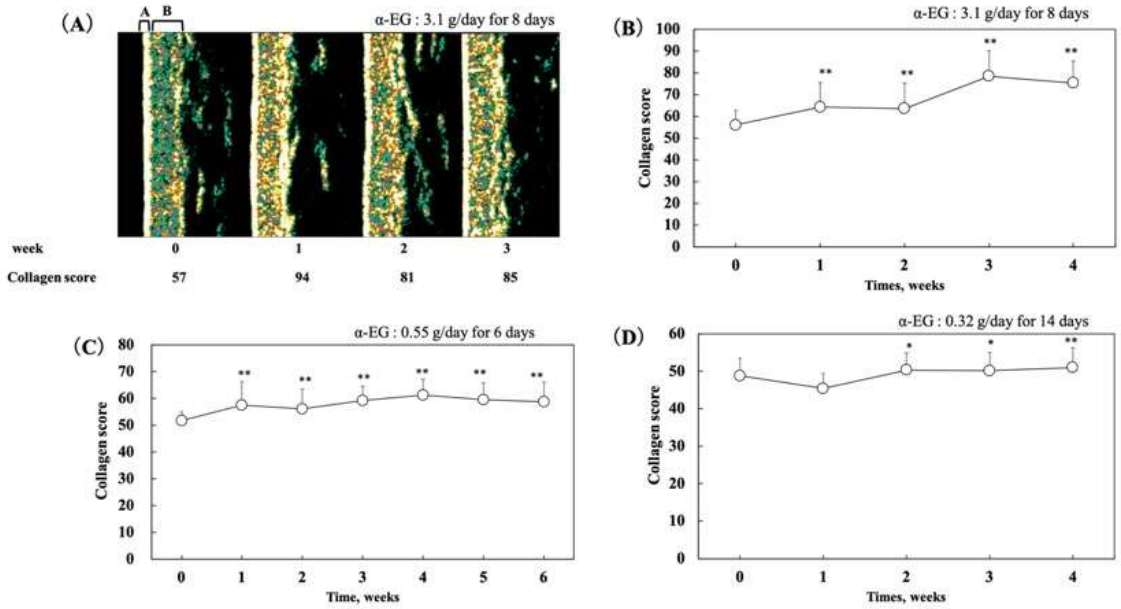

Fig. 2. Effect of sake containing α-EG on the collagen score.

(A) A 20-MHz ultrasonic echo images of the collagen content in skin after 1, 2 and 3 weeks of drinking sake containing 1.7% α-EG. The color scale of echogenicity is white>yellow>red>green>black. The symbols in the figure stand for epidermal A and dermis B. Representative images of the panelist 21 years old are shown. (B) Effect of α-EG on the collagen score by drinking 180 mL per day of sake containing 1.7% α-EG (α-EG content 3.1 g/day) during regular meals, prior to sleeping for eight days. Values are means SD of collagen score for all panelists (n=240). $**p < 0.01$ vs. 0 week value, dunnett test. (C) Effect of α-EG on the collagen score by drinking 50 mL per day of sake containing 1.1% α-EG (α-EG content 0.55 g/day) during regular meals, prior to sleeping for six days. Values are means SD of collagen score for all panelists (n=100). $**p < 0.01$ vs. 0 w values, dunnett test. (D) Effect of α-EG on the collagen score by drinking 40 mL per day of sake containing 0.8% α-EG (α-EG content 0.32 g/day) during regular meals, prior to sleeping for fourteen days. Values are means SD of collagen score for all panelists (n=240). $*p < 0.05$, $**p < 0.01$ vs. 0 week value, dunnett test.

Second experiment

In the second experiment, using sake with a lower concentration of α-EG at 1.1%, conducted by five 23-48 year-old female panelists, the collagen score increased by 6 points during the first week and reached the maximum of, 10 points, at the end of the fourth week

(Fig. 2C). Additionally we observed a significant increase in the collagen score after 5 weeks, even upon the termination of sake consumption. The results confirmed both immediate and long-term effects of α-EG in terms of collagen score increase.

Third experiment

In the third experiment, using *amazake* prepared from sake lees, conducted by four 40-59 year-old female panelists, the collagen score increased by 4 points during the first week, and further by 9 points (13 points in total) during the week after the cessation of s *amazake* consumption (Fig. 3A). A significant increase in keratinous moisture content was also observed, which was 3% during the first week and 8% during the week after the cessation of consumption (Fig.3B).

Fourth experiment

In the fourth experiment using sake with a lower concentration of α-EG (0.8%) conducted by eight 24-63 year-old female panelists, the collagen score increased by 1.5 points during the second week and reached the maximum, 2.2 points at the end of the fourth week. (Fig. 2D).

Discussion

In the first experiment, conducted by 21-22 year-old panelists, five out of the eight panelists showed a significant increase in the collagen score during the first week. The score continued to increase until the fourth week of the experiment, the cessation of sake intake. A 20-MHz ultrasonic echo image (Fig. 2A) showed an increase in the number of white and yellow dots, indicating enrichment of collagen fibers during the first week, which further continued even in the second and third week (after the termination of drinking). In the second experiment, conducted by 23-48 year-old female panelists, an immediate was observed in the collagen score for 6 days and a continuous increase for more than 1 month, even though the α-EG concentration in their drinks was less than 1/5 of that in the first experiment. Older panelists were more responsive than younger panelists, suggesting that collagen levels rapidly decrease between 30 and 39 years of age.[8] α-EG might also have a high capacity to activate fibroblasts and a long-term effect for more than 1 month. In the third experiment using *amazake*, the increase in the collagen score and keratinous moisture content in the second week after the cessation of sake consumption was larger than that in the

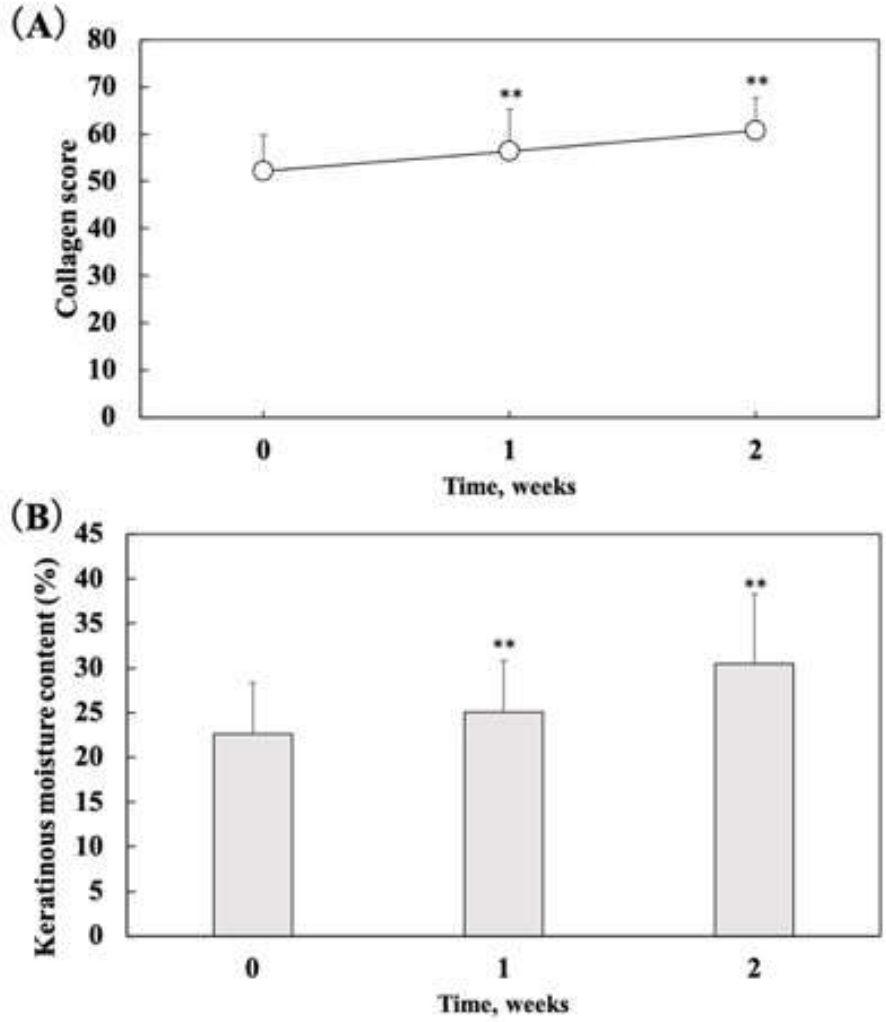

Fig. 3. Effect of sake lees containing α-EG on the collagen score and keratinous moisture content.

(A) Effect of α-EG on the collagen score by consuming 100 g of sake lees as *amazake* with α-EG content of 0.6% (α-EG content 0.6 g/day) for seven days as part of their normal diet. Values are means SD of collagen score for all panelists (n=120). $^{**}p < 0.01$ vs. 0 w values, dunnett test. (B) Effect of α-EG on the keratinous moisture content by consuming 100 g of sake lees as amazake with α-EG content of 0.6% (α-EG content 0.6 g/day) for seven days as part of their normal diet. Values are means SD of keratinous moisture content for all panelists (n=120). $^{**}p < 0.01$ vs. 0 week value, dunnett test.

first week. Since the daily intake of α-EG was almost the same between the two experiments using sake and sake lees (0.55 and 0.6 g/day, respectively), it can be assumed that sake lees has additional ingredients that maintain the effect of α-EG better. A questionnaire investigation carried out during the first week of and second week of sake consumption demonstrated that two panelists experienced bouncy skin and a good makeup appearance.

In the fourth experiment, conducted by 24-63 year-old female panelists, a significant effect was observed from the second week and was maintained into the third week (even after stopping to drink) in terms of high collagen density (α-EG: 0.32 g/day for 14 days). Further, significant increase in keratinous moisture content and collagen score (α-EG: 0.6 g/day for 7 days) was observed in third experiment using *amazake* prepared from 100 g of sake lees. It can be that collagen production could be kept activated once the accumulated amount of α-EG in the cells exceeded the effective concentration when the α-EG concentration was low. Therefore, even a consecutive consumption of 50 g/day of sake lees for 2 weeks is expected to increase collagen density and keratinous moisture content after two weeks. Long-term effects on the increase in collagen score have been reported for other fat-soluble substances as well,[9,10] although they increase the collagen score only after continuous intake for 1 month or longer. In contrast, α-EG significantly increased the collagen score, even in the first week of α-EG intake. To the best of our knowledge, α-EG is the most effective substance that affects collagen score even upon short-term intake.

α-EG consumed by drinking is absorbed into the bloodstream, transferred to the whole body, and passed to epidermal keratinocytes through skin capillaries.[4,6] Fibroblasts positioned closest to the capillaries are thought to be most closely involved in the production and secretion of collagen and in strengthening the collagen network that forms our skin foundation (Fig. 4). The significant increase in the collagen score and keratinous moisture content in our third experiment strongly suggests that α-EG rises to the stratum corneum and increases its moisture content. The average weight of adult males and females is approximately 64 kg and 50 kg,

respectively (National Health Survey 2015 and 2016, Ministry of Health, Labour and Welfare. Available at https://www.mhlw.go.jp/toukei/youran/indexyk_2_1.html (December 8, 2019)). Since a rough averages of blood approximately 8% of the body weight,[11] the blood volumes of males and females are 5.1 L and 4.0 L, respectively. When an adult with a blood volume of 5 L takes 1 g of α-EG, α-EG concentration in fibroblast cells is 10^{-4}%, assuming that the whole amount is transferred into the blood, and that 1/100 of the α-EG is absorbed into the fibroblast cells. In our cell experiments, a 1.6-times increase in collagen I, which was produced by NDHF, was observed at an α-EG concentration of 10^{-5}%, as compared to that in cells cultured without α-EG. Furthermore, the mRNA expression of fibroblast growth factor I and IV of NDHF cultured in α-EG supplemented medium increased by approximately 1.5-times at the α-EG concentrations of 4.8 as well as 0.048 μM.[7] These findings suggest that the oral uptake of α-EG, as described above, a direct effect on fibroblasts, resulting in proliferation and increased collagen production. Considering that the effects were observed immediately even with a small amount of α-EG intake, and that α-EG is one of the major ingredients in sake, higher resilience and better glow of the skin of Kurodo and Sumo wrestlers, who drink large amounts of sake, can be scientifically attributed to the intake of α-EG.

Several compounds, including vitamin C and marine collagen peptides, are known to increase the contents of collagen, elastic fibers and skin moisture.[12,13] However, the effect of these compounds appeared more than 2 weeks after intake thus, the reason α-EG takes less than 1 week to show its effects should be one of the main subjects to be addressed. It is also necessary to study the detailed mechanism by which the increased collagen levels in fibroblasts are maintained for more than 1 month, and the relationship between age and effective quantity. Measurement of α-EG concentration in the blood before and after α-EG intake is also important. While this report focuses on the collagen content measured in the inner side of both arms, it would be interesting to elucidate the effects of α-EG in the skin on the other parts and bones to understand the effect of α-EG on the whole body.

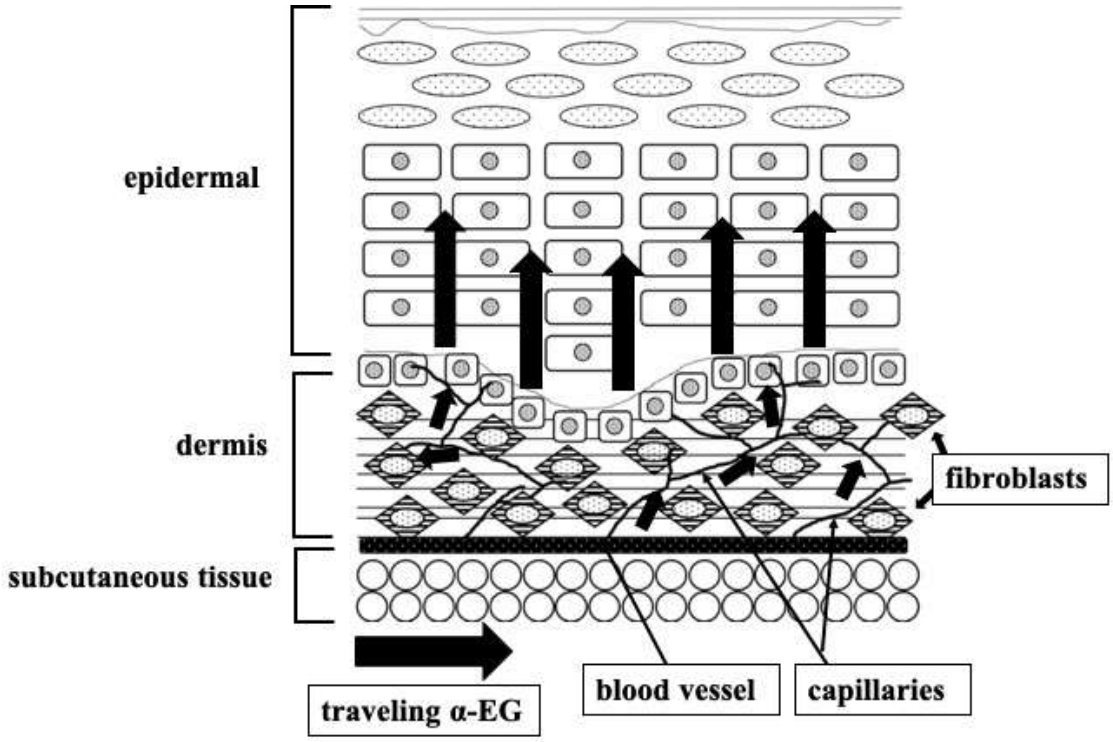

Fig. 4. Effect of α-EG intake on the dermis and epidermal.
α-EG taken by drinking is thought to be absorbed into bloodstream, transferred to whole body and passed to epidermal keratinocytes through skin capillaries. Fibroblasts positioned closest to the capillaries, are thought to be most closely involved in the production and secretion of collagen and to strengthen the collagen network which forms the skin foundation.

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