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Original Artcle

Hair protein glycation reduces hair breaking strength

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Abstract

***Objective*:** Glycation stress is a risk factor of aging in cells, tissue and organs, and causes both functional and morphological changes. However, few studies have examined the effects of advanced glycation end products (AGEs) on these changes, particularly in hair. Here, the content of AGEs in hair was measured to evaluate the effect of AGEs on hair breaking strength.
***Methods*:** Hair was glycated *in vitro* by incubation with 1 mol/L glucose in 0.1 mol/L phosphate buffer (pH 7.4) at 60°C for 5 days. Hair samples from 10 healthy Japanese female volunteers were collected from root (4 ~ 8 cm from the root) and peripheral regions (12 ~ 16 cm from the root). In addition, hair samples were obtained from 24 non-diabetic individuals between the ages of 20 and 90 (10 males and 14 females). The breaking strength and concentrations of fluorescent AGEs, pentosidine and protein were measured in the hair samples. All experiments were conducted under the approval of our institutional ethics committee.
***Results*:** The *in-vitro* glycation of hair reduced the protein content by 56% from the initial value of 0.28 ± 0.09 to 0.11 ± 0.04 mg/mg hair in 5 days and also decreased the breaking strength by 73%, from 27.5 ± 4.4 to 20.2 ± 2.5 kgf/mm². The amount of fluorescent AGEs per mg protein increased by 215%, from 2.5 ± 0.5 to 5.4 ± 1.7 counts/mg protein, and pentosidine levels increased by 632%, from 0.08 ± 0.10 to 0.52 ± 0.19 ng/mg protein. Fluorescent AGEs and pentosidine were more abundant in the peripheral regions of hair than those in the root region. Correlation analysis showed that the protein content of hair declined with age (r = -0.578)and that fluorescent AGEs increased (r = 0.449). Hair care treatment such as hair dye enhanced these effects.
***Conclusion*:** Several properties of hair, particularly a reduction of hair protein content, increase in AGEs, and lowered breaking strength, may be involved in the sclerotic changes that occur in hair with aging.

***KEY WORDS*:** hair, advanced glycation end products (AGEs), pentosidine, breaking strength

Introduction

In the body, reducing sugars and aldehydes react non-enzymatically with protein N-terminal amino groups, such as lysine and arginine, leading to the formation of advanced glycation end products (AGEs). AGE formation reactions proceed irreversibly in an uncontrolled manner and cause various conformational and optical changes, in addition to cross-linkage formation, in the AGE-modified proteins. The production and accumulation of AGEs increase with aging and play a role in the pathogenesis of diabetes and aging-related diseases, such as cataracts and vascular atheloscrerosis. For this reason, AGEs are considered to be a serious risk factor of aging [1-6].

Hair is produced from the base of hair follicles in the connective tissue of skin through the repeated cell division of hair stem cells. The produced daughter cells undergo keratinization while moving toward the cutaneous surface, where they produce the proteins that form hair. Because hair is exposed to the external environment, it is subject to various stresses, including ultraviolet (UV) light and cosmetic procedures, such as hair dye or permanent wave treatment, which are reported to alter the properties of hair proteins [7-10]. For example, UV exposure increases the fragility of hair, making it more susceptible to breakage and sclerotic changes [11,12]. Changes in the properties of hair have also been utilized as indices of environmental exposure and disease. For example, the AGE composition of hair has been studied as an index for the diagnosis of diabetes mellitus. In particular, the concentrations of frosine [13-15] and fructosamine [16] in the hair of diabetic patients positively correlate with serum HbA1c levels. However, no studies have examined the effects of AGEs on the properties of hair.

In the present study, we hypothesized that hair protein glycation alters the properties of hair, similar to that observed in skin and bone, and cause deterioration of hair quality.

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To evaluate this hypothesis, here, the effect of hair protein glycation on hair breaking strength was examined using an *in vitro* model of hair protein glycation. In addition, the concentrations of AGEs were measured in different regions of hair strands collected from a wide age range of men and women.

Methods

Materials

Hair samples were collected from 10 men and 24 women by cutting individual hairs at less than 5 mm from the scalp. For each person, 10 or more individual hair samples were collected from the occipital and temporal regions of the head. Whole hairs that fell out by brushing were also collected and were cut at less than 5 mm from the hair root. The location of the cut was designated as 0 mm. Hair samples were placed into a 2-l airtight container containing 100 g silica gel and stored in the dark at 4°C until used for analysis.

Using a questionnaire, information about the subjects' history of hair cosmetic procedures, such as hair dye or permanent wave treatment, in the past two years was collected.

Sample preparation

(1) *In-vitro* glycation of hair

Five ~150-mg bunches of hair were prepared from 20-cm lengths of hair from a healthy woman in her 30's. A 0.125% sodium laureth sulfate solution was prepared for use as a cleaning fluid, as previously described [17]. The hair samples were added to 100 mL cleaning fluid in a container and were then shaken for 1 min. After removing the cleaning fluid, tap water was added into the container, which was further shaken for 1 min. The tap water was replaced with fresh tap water and the container was again shaken for 1 min. The washing step was repeated three more times. After wiping excess water from the hair surface, the hair samples were dried at room temperature for more than 1 h. One bunch of hair was stored at 4°C (untreated hair). Two bunches of hair were soaked in 0.1 mol/L phosphate buffer (pH 7.4) containing 1 mol/L glucose and incubated at 50°C for either 40 h or 5 days (G(+)hair). The remaining two bunches of hair were soaked in 0.1 mol/L phosphate buffer (pH 7.4) and incubated at 50°C for 40 h and 5 days as control samples (G(-)hair). After the incubations, all hair samples were washed five times with tap water and then dried at room temperature for more than 1 h.

(2) Samples of root and peripheral areas of hair strands

Hair bunches of ~150 mg (approximately 200 ~ 300 hairs) were prepared using 20-cm lengths of hair (including the root) collected from 10 healthy women in their 20 ~ 30's (mean age, 24.6 ± 4.9 years). Six of the women had had cosmetic treatments (hair dye [n = 4] and permanent wave [n = 2]) within the past two years. After measuring the hair breaking strength, as described below, the root (4 ~ 8 cm from the hair root) and peripheral areas (12 ~ 16 cm from the hair root) of the hair strands were cut out from each bunch of hair.

(3) Hair samples from different aged individuals

Hair bunches of ~40 mg were prepared using 3-cm lengths of hair collected from 24 non-diabetic men (n = 10) and women (n = 14) in their 20 ~ 80's. The age distribution of the subjects was: 20's (n = 5; 3 men, 2 women), 30's (n = 5; 2 men, 3 women), 40's (n = 3; 1 man, 2 women), 50's (n = 4; 2 men, 2 women), 60's (n = 1; woman), 70's (n = 5; 2 men, 3 women), and 80's (n = 1; woman). The 24 subjects were grouped according to sex and history of cosmetic hair treatment (hair dye and permanent wave). A total of 13 subjects did not have any type of cosmetic treatment within the past 3 months. Eleven of the subjects had dyed their hair and 5 had permed their hair within the past 2 years.

Measurement of hair breaking strength

The breaking strength of the *in-vitro* glycated hair and the hair samples of peripheral and root regions was measured, as described as below. The hair samples collected from individuals with the different age groups were not measured due to a shortage of sample material. Each sample was incubated at 23 ~ 24°C and 35% ~ 38% humidity for 2 h. The diameter of *in-vitro* glycated hair was measured using a hair diameter measuring instrument (Hahoniko, Tennouji-ku, Osaka, Japan) at 3 sites, 2, 6 and 10 cm from the root, and that for the other hair samples was measured at 5 sites, 2, 6, 10, 14, and 18 cm from the root. The cross-sectional area (mm^2) of hair was calculated based on the following equation (1), which assumes that the hair was circular in diameter:

$$\text{Area of hair cross section } (mm^2) = (\text{Average diameter of 3 sites } (mm)/2)^2 \times \text{Circle ratio} \quad (1)$$

The breaking strength (gf) of hair samples was measured using a hair analyzer (Hahoniko). *In-vitro* glycated samples were affixed between the fixation and winding clamps of the apparatus at sites 4 ~ 8 cm from the root. The root and peripheral hair samples were measured at 4 ~ 8 cm and 12 ~ 16 cm away from the root, respectively. After affixing the hair samples, the hair strand was pulled in the direction of the long axis at a rate of approximately 180 mm/min. The measurement was conducted on 20 hair samples from each person, and the breaking strength (kgf/mm^2) was then calculated using the following equation (2):

$$\text{Breaking strength } (kgf/mm^2) = \text{Breaking weight } (gf) \times 10^{-3} / \text{Area of hair cross section } (mm^2) \quad (2)$$

Purification and measurement of hair protein

Each hair sample was immersed in a degreasing agent (chloroform/methanol (MeOH) = 2/1) at room temperature for 16 h. The hair was removed from the degreasing agent, dried at room temperature for > 1 h, and then cut into lengths of < 5 mm. Ten milligrams of each hair sample were added into 500 µL extraction buffer, which was composed of 30% urea, 20% thiourea, and 10% 2-mercaptoethanol in 20 mmol/L sodium tetraborate buffer (pH 9.5), as described previously [18], and then incubated at 50°C for 2 days under stirring (n = 3). The sample was then centrifuged at 10,000 rpm for 1 min at 20°C and the resulting supernatant (225 µL) was mixed with 2 mL distilled water and 750 µL of 7% perchloric acid (PCA) under stirring. The solution was allowed to stand for 20 min, and was then centrifuged at 10,000 rpm for 15 min at room temperature. After the supernatant was removed, 2 mL of 1.75% PCA was added under stirring to wash the obtained pellet. The sample was further centrifuged at 10,000 rpm for 1 min at 20°C. The supernatant was removed and 2.25 mL of 20 mmol/L sodium

tetraborate buffer (pH 9.5) was added under stirring. After centrifugation of the mixture at 10,000 rpm for 1 min at 20°C, the obtained supernatant was collected as protein sample.

Into each well of a 96-well microplate (n = 2), 5 µL protein solution was added, followed by 25 µL A solution and 200 µL B solution from a DC Protein Assay kit (Bio-Rad, Hercules, CA, USA). After a 30-min incubation at room temperature, absorbance at 750 nm was measured using a spectrophotometric microplate reader (SPECTRA MAX 190; Molecular Devices, Sunnyvale, CA, USA). Protein content was calculated by comparing the average absorbance values of two wells to a standard curve generated using keratin (Nacalai Tesque, Nakagyo-ku, Kyoto, Japan).

Measurement of fluorescent AGEs in hair protein

Fluorescent AGEs in hair protein were measured using a previously reported method [19]. Briefly, samples of hair protein fluid (200 µL each) were injected into wells on microplates (n = 2). Fluorescent AGEs in samples were measured using a microplate reader (ARVO MX 1420, ARVO series Multilabel Counter; Perkin-Elmer, Yokohama, Kanagawa, Japan) and excitation and emission wavelengths of 370 and 440 nm, respectively. Fluorescence intensity values are expressed as relative counts compared to the assumed value of 1,000 in a 200-µL solution of 0.1 N sulfuric acid containing 5 µL/mL quinine.

Measurement of hair protein pentosidine

The pentosidine content in hair protein of *in-vitro* glycated hair and hair samples of peripheral and root regions was measured by method (a) (described below) using an ion-exchange column (Oasis MCX; Waters, Milford, MA, USA), which was modified from a previous report [20]. The pentosidine content of hair samples from the different age groups was measured by method (b) (described below) using filter paper powder A (Advantec Toyo, Tokyo, Japan). The same samples (n = 3) were used for measurements by both methods (a) and (b) to examine differences in methodology. In addition, the recovery of pentosidine by method (b) was evaluated by adding0.028 ng purified pentosidine (PPL-FR, Strasbourg, France) to randomly collected hair samples (n = 4), and the total amount of pentosidine was then measured, followed by calculation of the recovery ratio (%).

Method (a): Each protein sample (500 µL) was hydrolyzed in 1 mL of 6 N HCl by incubation at 100°C for 18 h. HCl was removed using a centrifugal evaporator, and the resulting residue was dissolved in 1 mL distilled water (hydrolyzed sample). Methanol (MeOH) and distilled water (1 mL each) were passed through an Oasis MCX column, which was then loaded with 800 µL of the hydrolyzed sample. The column was washed with 3 mL 0.1 N HCl, and pentosidine was eluted by adding 4.5 mL of 7% NH_3 solution. After the eluent was collected, NH_3 was evaporated to dryness, and the obtained residue was dissolved in 240 µL high-performance liquid chromatography (HPLC) eluent A, which was comprised of 16% acetonitrile (ACN), 4% methanol and 0.2% heptafluorobutyric acid (HFBA). The sample mixture was then analyzed by HPLC using the following analytical conditions: separation column: YMC Triart C18 (column size 150 × 4.6 mm ID, 5 µm/12 nm; YMC, Shimogyo-ku, Kyoto, Japan), flow rate: 1.0 mL/min, and column temperature: 30°C. The samples were detected using an excitation wavelength of 335 nm and a detection wavelength of 385 nm. Elution was performed using eluent A (ACN/MeOH/water/HFBA = 16/4/76/0.2) and eluent B (ACN /HFBA = 100/0.2), and the following gradient conditions: analysis: 0 ~ 20 min with 0% eluent B; washing: 20 ~ 25 min with 100% eluent B 100%; and conditioning: 25 ~ 40 min with 0% eluent B. A standard curve was generated using standard pentosidine samples (PPL-FR).

Method (b): Each protein sample (500 µL) was hydrolyzed in 500 µL of 6 N HCl by incubation at 100°C for 18 h. A slurry was prepared by mixing 1-butanol, acetic acid and 6 N HCL (8/1/1, v/v), followed by the addition of 5.26% (w/v) filter paper powder A. A column wash solution was prepared by mixing 1-butanol, acetic acid and distilled water (8/1/1, v/v). A 4% acetic acid solution was prepared as the eluent. The sample mixture solution consisted of 8 mL 1-butanol, 1 mL acetic acid, 1 mL slurry and 3.2 mL of hydrolyzed protein solution (n = 3). To pretreat the column, 8 mL slurry was added to a disposable reaction tube, through which the above eluent was passed. The total amount of the prepared sample mixture was injected into the pretreated column, and the eluent was then passed through column. The container tube, in which samples were prepared, was washed with 30 mL column washer solution, which then was then injected into the pretreated column. A 4% acetic acid solution (4.5 mL) was then injected into the column to elute the sample. The movement of solvent was facilitated using decompression suction (-0.02 MPa).

The organic layer that formed in the upper layer of the eluent was removed. The remaining water layer was dried and fixed using a centrifugal evaporator. The obtained debris was dissolved in 240 µL HPLC eluent A, and the sample was then analyzed by HPLC. The analytical conditions were the same as those used in method (a). The pentosidine content per mg protein in hair samples was reported as 0 ng/mg protein if the measured value was less than the limit of quantitation of the assay (0.02 ng/mg protein).

Statistical analysis

Tukey's HSD test was used to identify differences between glycated hair samples from the five groups. The Student's t test was used to compare data between basal and peripheral regions of hair. Pearson's analysis was used to analyze the relationship between age and measured data for each age group. The Mann-Whitney U test was used for inter-group analysis. Data are expressed as means ± standard deviation. All statistical analyses were performed with SPSS II software (IBM Japan, Minato-ku, Tokyo, Japan). The significance level was set at $p < 0.05$ for the two-sided test.

Ethical standards

Prior to providing hair samples, informed written consent was obtained from each subject. The present study was conducted under in accordance with the study protocol approved by the ethics committee of Doshisha University (Approval number #1270).

Results

Properties of in-vitro glycated hair samples

Fig. 1 shows the results of tests for breaking strength and protein content of control [G(-)] and *in-vitro* glycated [G(-)] hair. The breaking strength of G(+)hair treated for 5 days (20.2 ± 2.5 kgf/mm²) was significantly lower than that of untreated

hair (27.5 ± 4.4 kgf/mm², $p < 0.001$). The breaking strength of G(-)hair exposed to treatment buffer for 40 h (23.3 ± 3.9 kgf/mm², $p = 0.003$) and 5 days (24.0 ± 2.8 kgf/mm², $p = 0.021$) was also significantly lower than that untreated hair. In addition, the breaking strength of G(+)hair treated for 5 days was significantly lower than that G(-)hair exposed to treatment buffer for 5 days ($p = 0.008$, Fig.1-a).

The protein content of G(+)hair treated for 40 h (0.16 ± 0.03 mg/mg hair, $p < 0.001$) and 5 days (0.11 ± 0.04 mg/mg hair, $p < 0.001$) was significantly lower than that of untreated hair (0.28 ± 0.09 mg/mg hair). The protein content of G(-) hair exposed to treatment buffer for 5 days (0.15 ± 0.03 mg/mg hair, $p < 0.001$) was also significantly lower than that of untreated hair. However, the protein content of G(+)hair samples treated for 40 h were significantly lower than those of G(-)hair exposed to treatment buffer for 40 h ($p = 0.005$), although no significant differences were detected between the 5-day treated samples of G(+)hair and G(-)hair (Fig.1-b).

The breaking strength per mg protein of G(+)hair after 40 h ($p < 0.001$) and 5 days of treatment ($p < 0.001$) was significantly higher than that of untreated hair ($p < 0.001$). Although no significant differences in breaking strength between G(-)hair exposed to treatment buffer for 40 h and untreated hair were detected, the breaking strength of G(-)hair exposed to treatment buffer for 5 days was significantly higher than that untreated hair ($p < 0.001$). After 40 h and 5 days of treatment or buffer exposure, the breaking strength of G(+) hair was significantly higher than that G(-)hair under both conditions ($p < 0.001$, Fig.1-c).

Regarding the content of hair AGEs, the amount of fluorescent AGEs per mg protein of G(+) hair treated for 40 h (4.8 ± 1.9 counts/mg protein, $p < 0.001$) and 5 days (5.4 ± 1.7 counts/mg protein, $p = 0.002$) was significantly higher than that of untreated hair (2.5 ± 0.7 counts/mg protein), but did not significantly differ between the two treatment durations. The amount of fluorescent AGEs per mg protein of G(-)hair did not significantly differ from that of untreated hair, but was significantly lower than that of G(+)hair treated for either 40 h ($p < 0.001$) or 5 days ($p = 0.002$, Fig.1-d).

The pentosidine content of G(+) hair treated for 40 h (0.38 ± 0.08 ng/mg protein, $p = 0.003$) and 5 days (0.52 ± 0.19 ng/mg protein, $p < 0.001$) was significantly higher than that of untreated hair (0.08 ± 0.10 ng/mg protein), but did not significantly differ between the two treatment durations. No marked difference was observed between the pentosidine content of the two groups of G(-)hair. However, the pentosidine content of G(+)hair was significantly higher than that G(-)hair for both the 40 h ($p < 0.001$) and 5 day samples ($p < 0.001$, Fig.1-e).

Properties of hair samples from different regions

Whole hair samples were divided into peripheral and root regions to compare the breaking strength, and protein and AGE contents between these regions (***Fig.2***). Regarding the breaking strength and protein content, no significant differences were detected between the root and peripheral regions (Fig.2-a,b). However, the breaking strength per mg protein of the root region was higher than that of the peripheral site, although the difference was not significant ($p = 0.084$, Fig.2-c). The content of fluorescent AGEs in hair samples from the peripheral region (1.9 ± 0.7 count/mg protein) was significantly higher than that in samples from the root region (1.1 ± 0.6 counts/mg protein, $p < 0.001$, Fig.2-d). Similarly, the pentosidine content of the peripheral region (0.20 ± 0.07 ng/mg protein) was also significantly higher than that of the root region (0.10 ± 0.08 ng/mg protein, $p = 0.007$, Fig.2-e).

The protein and fluorescent AGE contents of hair exposed to cosmetic treatment were compared to the values for non-treated hair. The comparison revealed that non-cosmetic–treated hair had lower values for both of these parameters (root, 0.26 ± 0.13 ng/mg protein and 0.7 ± 0.1 counts/mg protein; peripheral, 0.23 ± 0.08 ng/mg protein and 1.4 ± 0.1 counts/mg protein) than treated hair (root, 0.43 ± 0.06 ng/mg protein, $p = 0.067$, and 1.3 ± 0.7 counts/mg protein, $p = 0.067$; peripheral, 0.33 ± 0.03 ng/mg protein, $p = 0.019$, and 2.3 ± 0.7 counts/mg protein, $p = 0.067$; Fig.2-b,d). In addition, the breaking strength of cosmetic-treated hair (root, 1.3 ± 0.7 kgf/mm²/mg protein; peripheral, 2.3 ± 0.7 kgf/mm²/mg protein) was also higher than that of non–treated hair (root, 0.7 ± 0.1 kgf/mm²/mg protein, $p = 0.171$; peripheral, 1.4 ± 0.1 kgf/mm²/mg protein, $p = 0.010$, Fig.2-c) although the difference was only significant for the peripheral region.

Effects of hair age on protein, pentosidine and AGE content

Table 1 shows a comparison of the measurements of protein, pentosidine, and AGE contents in hair samples grouped according to sex (men, women), hair dye treatment (with, without), and permanent wave treatment (with, without). The pentosidine content of hair samples measured using cellulose powder A was 0.9-fold lower than that measured using an Oasis MCX ion-exchange column by Method (b). The recovery rate of pentosidine from hair samples using cellulose powder A was 50%. No significant differences were detected between the three examined parameters with respect to samples grouped according to sex or permanent wave treatment. However, the protein content of samples with hair dye was 0.6-fold lower than that found in samples without hair dye ($p = 0.021$). In addition, the amount of fluorescent AGEs per mg protein was 1.6-fold higher in samples with hair dye than those without ($p < 0.001$).

Fig.3-a~c shows the measured values of the three examined parameters with respect to age. In Fig.3-a, regression lines for the protein content of all hair samples (solid line, $n = 24$) and samples without hair dye (dotted line, $n = 16$) are shown. The protein content significantly decreased with increasing age regardless of the presence or absence of hair dye (Fig.3-a).

The content of fluorescent AGEs per mg protein significantly increased in all hair samples with increasing age (Fig.3-b). However, in contrast to the samples without hair dye treatment, the correlation between the fluorescent AGE content and age was not significant. No significant correlation between age and usage of hair dye was found ($p = 0.263$).

With respect to pentosidine content, no correlation was detected between age and pentosidine content per mg protein due to the large individual differences that were observed in each age group. In addition, no significant correlation between the pentosidine content per mg protein and hair dye treatment was detected., and sex and permanent wave exposure did not show a relationship with each other.

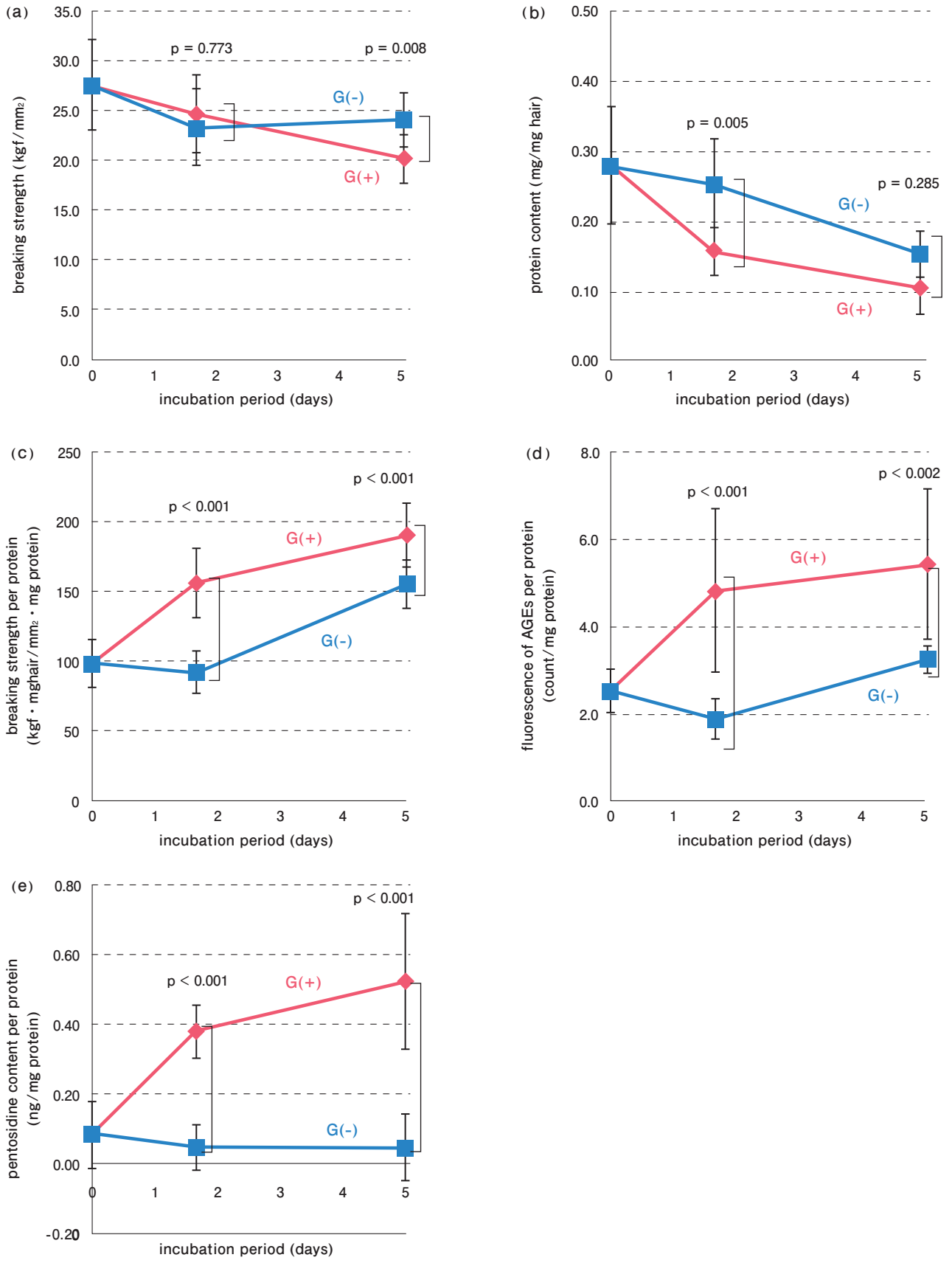

Fig.1. **Effects of glycation on properties of hair.**
(a) Breaking strength, (b) protein content, (c) breaking strength per mg protein, (d) fluorescent AGE content, and (e) pentosidine content. All values are shown as the mean ± standard deviation. p values between G(+)hair and G(-)hair are shown (Tukey's HSD test, n = 10).

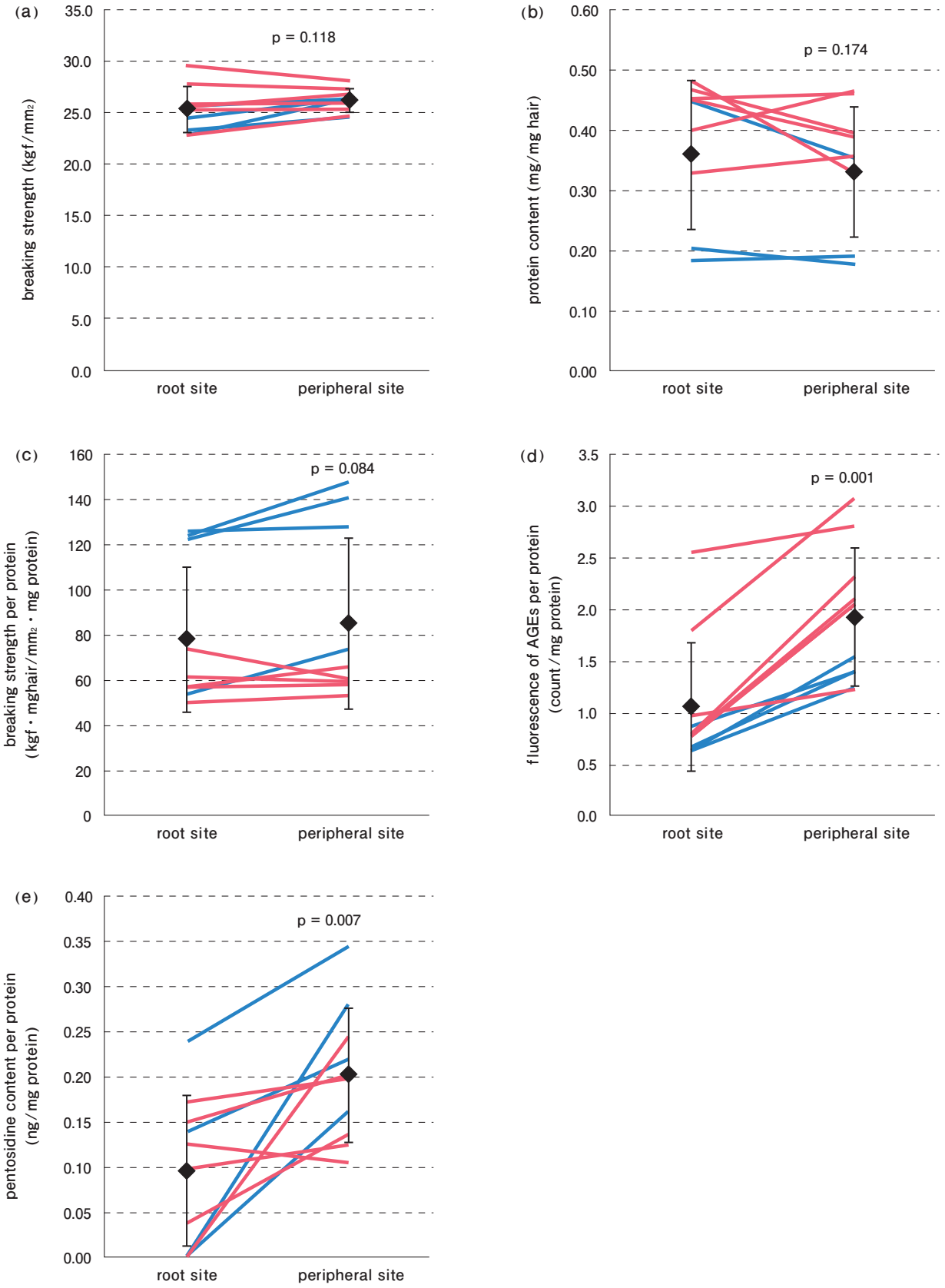

Fig.2. **Comparisons between root and peripheral regions of hair for**
(a) breaking strength, (b) protein content, (c) breaking strength per mg protein, (d) fluorescent AGEs, and (e) pentosidine content.
Blue line: without cosmetic treatment (n = 4), Red line: with cosmetic treatment (n = 6).
Mean ± standard deviation (◆)(paired t-test, n = 10).

Table 1. Fluorescent AGE and pentosidine contents of hair based on sex and hair treatment.

Parameter	Sex			Hair dye			Permanent wave		
	Male (n = 10)	Female (n = 14)	p value	With (n = 8)	Without (n = 16)	p value	With (n = 5)	Without (n = 19)	p value
Protein content (mg/mg hair)	0.19 ± 0.08	0.21 ± 0.09	0.472	0.14 ± 0.03	0.23 ± 0.08	0.021	0.18 ± 0.07	0.20 ± 0.08	0.534
Fluorescent AGE content (counts/mg protein)	2.8 ± 1.0	2.9 ± 1.0	0.796	3.8 ± 0.7	2.3 ± 0.7	<0.001	3.2 ± 0.8	2.7 ± 1.0	0.208
Pentosidine content (ng/mg protein)	0.02 ± 0.02	0.02 ± 0.01	0.625	0.02 ± 0.02	0.02 ± 0.02	0.534	0.02 ± 0.02	0.02 ± 0.02	0.891

Mean ± standard deviation, Mann-Whitney U test.

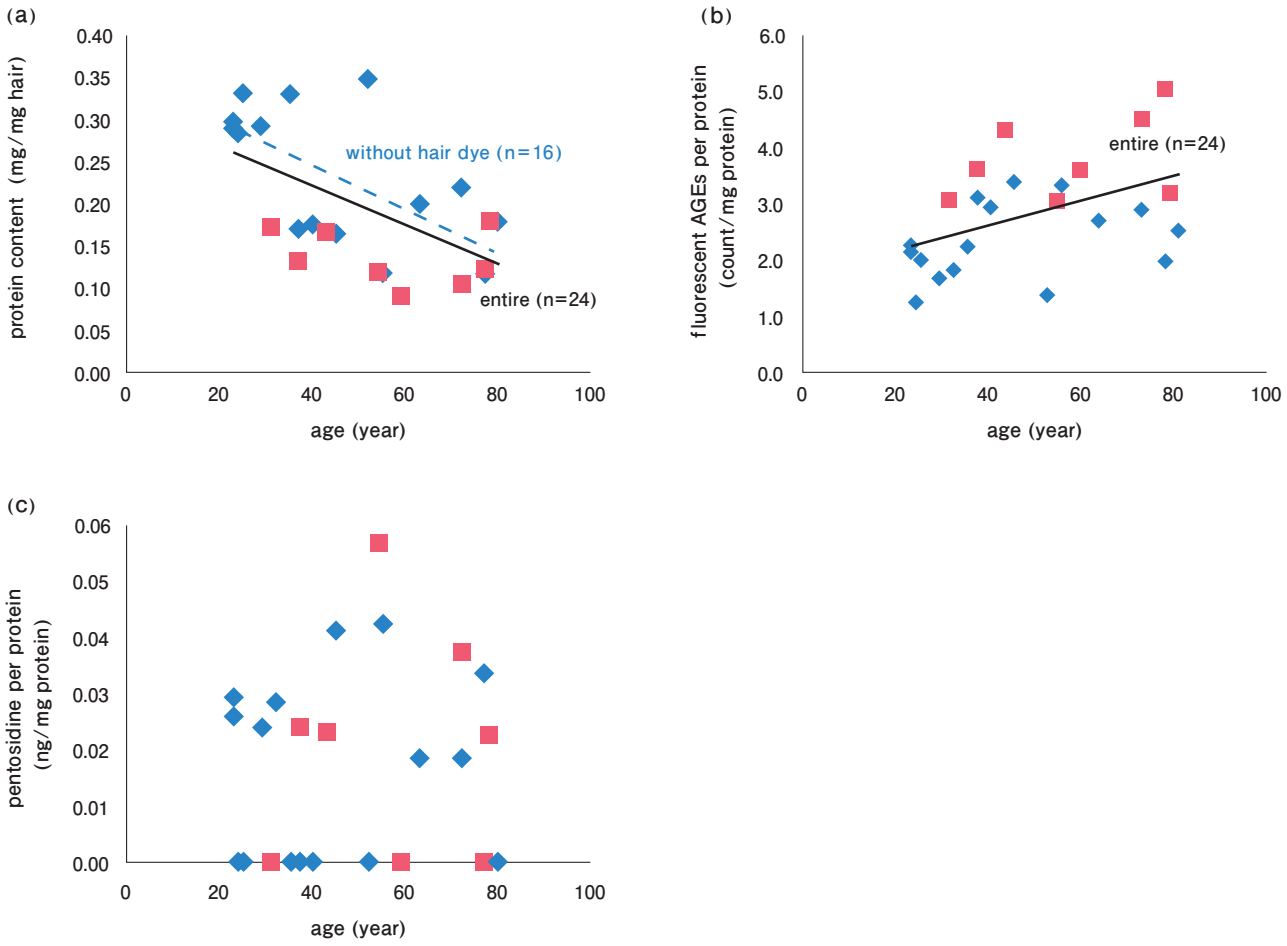

***Fig.3.* Protein, AGE, and pentosidine content in hair according to age.**

◆ : without hair dye (n = 16), ■ : with hair dye (n = 8).

(a) protein content

Solid line: all samples, y = −0.0023x + 0.315 (r = −0.578, p = 0.003)

Dotted line: without hair dye, y= −00.0026x + 0.348 (r = −0.657, p = 0.006)

(b) fluorescent AGE content per mg protein

Solid line: all samples, y = 0.022x + 1.76 (r = 0.449, p = 0.028)

(c) pentosidine content per mg protein

Discussion

Hair protein glycation and breaking strength

In the present study, the measured indices of protein content and breaking strength decreased time-dependently in glycated hair samples. Regarding AGEs, the amount of fluorescent AGEs and pentosidine per mg protein increased in hair samples incubated with glucose. This finding is consistent with the reported time- and glucose-concentration-dependent increase of fructosamine in hair [16]. The changes of breaking strength differed between the analyses of whole hair samples and those performed on a per protein basis; specifically, the former decreased, whereas the latter increased. Taken together, the findings from our *in-vitro* hair glycation study indicate that the protein content and breaking strength of hair are reduced over time, and that AGE-modified proteins accumulate as hair ages.

Pentosidine forms non-physiological crosslinkages between collagen fibers in the skin [3] and bone [4,5], and therefore contributes to reduced skin elasticity [6] and bone quality [5]. In G(+)hair, the pentosidine content, breaking strength, and fluorescent AGEs per mg protein increased compared with the levels in untreated hair. Thus, glycation may promote crosslinkage formation in hair protein, resulting in sclerotic changes. Scott et al. [11,12] measured hair winding strength as an index of sclerotic change and found that sclerotic hair has increased resistance to styling. It is therefore possible that similar changes occur in older hair, which has higher fragility and stiffness, and is also resistant to styling. Because the winding strength of hair samples was not examined in the present study, the relationship between hair glycation and resistance to styling treatment could not be confirmed.

Hair AGEs in root and peripheral areas

We demonstrated that both free AGEs and AGE-modified proteins in hair samples are more abundant in peripheral regions than in root regions. Thus, hair glycation appears to proceed from the root and increase in the periphery of hair strands with time. Oimomi et al. [13] and Yajima et al. [14] reported that hair frosine content is positively correlated with serum HbA1c levels in diabetic patients and speculated that a past history of high plasma glucose may affect hair protein AGE content. Because HbA1c was not evaluated in the present study, the correlation between hair AGE content and past glucose history could not be clarified. However, the amount of hair AGEs per mg protein was two-fold higher in peripheral regions (12 ~ 16 cm from the root) than that in the root regions (4 ~ 8 cm from the root) of hair strands obtained from healthy young women between the ages of 20 and 39. The reason for this difference may be due to the increased accumulation of hair protein AGEs in older regions of hair strands, a process that may be dependent on glucose history.

Monosaccharides are not typically used in commercial hair care products for hair coloring, permanent wave treatment, shampooing, or styling. With the exception of honey, sugars are not used to treat hair during normal cosmetic procedures. However, because sweat contains 0.03 ~ 0.5 mg/mL saccharides [21] it is possible that sweat provides reducing sugars that are involved in hair protein glycation. Other external factors that promote the glycation reaction include exposure to heat, as glycation is a non-enzymatic reaction whose rate largely depends on temperature, and UV light, which also promotes the formation of free radicals and reactive oxygen species in hair. For example, UV exposure is reported to enhance CML generation in skin keratin [22]. Thus, we speculate that AGE generation may be enhanced in the peripheral regions of hair, which depending on its length, may have had long-term exposure to UV and heat damage from hair dryers.

Hair AGEs and aging

In the analysis of the correlation between age and AGE content of hair protein, we found that the concentration of fluorescent AGEs per hair mg protein tended to increase with older age. However, in hair not treated with dye, a correlation between these factors was not detected, suggesting that hair treatment such as coloring may have a more significant impact on the accumulation of AGE in hair than aging. It is possible that hydrogen peroxide, which is used for hair coloring, may augment AGE formation by oxidative reactions, which are known to enhance glycation. However, it is difficult to rule out the possibility that hair-coloring treatment itself may affect the fluorescence intensity of AGEs. Further studies are needed to clarify the relation between hair glycation and coloring treatment.

The present results also show that the protein content of hair tends to decrease with advanced age. This finding may be related to decreased protein synthesis, as expression of the keratin gene in hair follicles is reported to decline with age [23], a response that is compatible with our present results. Thus, it is possible that the reduced protein content of hair in the elderly may be associated with a higher frequency of split hairs and reduced hair breaking strength.

It is worth noting that in a large number of hair samples, the pentosidine content was below the limit of detection. The pentosidine content may have been low in these hair samples because they were obtained from areas close to root and had only been exposed to the environment for a few months. Assays that are able to detect pentosidine with high sensitivity are needed to confirm this speculation.

Conclusion

The present study has shown that several changes in hair quality, particularly a reduction in protein content, increase in fluorescent AGE and pentosidine content per mg protein, and decreased hair breaking strength, may be involved in the sclerotic changes that occur in hair with aging. However, because the relationship between hair AGE content and age could not be conclusively demonstrated, further studies examining the effects of these hair properties by tests other than breaking strength are needed to elucidate how glycation affects hair quality.

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Conflicts of interest statement

The authors have no conflicts of interest related to this study to declare.

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