

ORIGINAL ARTICLE

The Ethnic Differences of the Damage of Hair and Integral Hair Lipid after Ultra Violet Radiation

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Background: Genetic factors account for the majority of differences in skin color and hair morphology across human populations. Although many studies have been conducted to examine differences in skin color across populations, few studies have examined differences in hair morphology.

Objective: To investigate changing of integral hair lipids after ultraviolet (UV) irradiation in three human ethnic groups.

Methods: We studied the UV irradiation induced hair damage in hairs of three human populations. UV irradiation had been performed with self-manufactured phototherapy system. Damaged hair samples were prepared at 12 and 48 hours after UVA (20 J/sec) and UVB (8 J/sec) irradiation. We evaluated the changes of hair lipid using scanning electron microscopy (SEM), transmission electron microscopy (TEM), lipid TEM and HP-TLC. After UV irradiation, hair surface damage was shown. **Results:** African hair showed more severe damage on hair surface than others. The lipid compositions across human populations were similar, but Asian hair had more integral hair lipids than other groups as a whole. Especially, free fatty acid contents were higher than other lipids. After UV irradiation, lipid contents were decreased. These patterns were shown in all human populations. Asian hair has more integral hair lipid than

European or African hair. After UV irradiation, European and African hair samples exhibited more damage because they have less integral hair lipids. However, Asian hair samples have less damage. **Conclusion:** We conclude that integral hair lipid may protect the hair against the UV light. (**Ann Dermatol 25(1) 54 ~ 60, 2013**)

-Keywords-

Ethnic groups, Integral hair lipid, UV irradiation

INTRODUCTION

Genetic factors account for the majority of differences in skin color and hair morphology across human ethnic groups. Although many studies have been conducted to examine differences in skin color across populations, few studies have examined differences in hair morphology. However, interest in geographical variations in hair has increased in regards to the cosmetics industry. Previous studies confirmed both morphological and biochemical differences across ethnic groups. Morphological studies have focused mainly on hair color, shape, and responses to external stimuli, while biochemical studies have focused on the compositions of the hair proteins. Only a few studies have assessed the differences in hair lipids across groups. This may be due to the chemical composition of hair, which is composed mainly of keratin protein. Additionally, there are few established methods to examine lipids in human hair.

Hair lipids are composed of fatty acids, cholesterol sulfate, ceramides, and cholesterol. Together, these account for 0.7 ~ 1.3% of the total chemical content of hair^{1,2}. Integral hair lipids are located in the cell membrane complex

Received July 27, 2011, Revised November 30, 2011, Accepted for publication December 14, 2011

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(CMC) of hair cuticles, and are important in the maintenance of hair integrity due to qualities including hydrophobicity, moisturization, and stiffness^{1,3}. Integral lipids of the hair and epidermal lipids of the skin have similar barrier functions; however, little is known about the nature of population differences in the production of integral hair lipids¹.

Forms of hair damage include physical stress, such as shampooing and combing, and chemical stress, like dyeing and bleaching. The most damaging among these factors is continuous exposure to sunlight. Ultraviolet (UV) light-induced hair damage is difficult to avoid^{4,5}. Continuous UV-light exposure results in dryness, roughness, sun-bleaching and breakage due to photo-oxidation⁶.

In this study, we examined integral hair lipids in three human populations. The results of UV-induced integral hair lipids differed across groups.

MATERIALS AND METHODS

Materials

Standardized (age and sex matched) hair samples from individuals originating from three different geographical ethnic groups (Asian, European, and African) were purchased from De Meo Brothers, New York, NY, USA. The hair samples (hair tresses) were washed with a 3% diluted commercial shampoo, and then rinsed thoroughly with water and dried in ambient conditions⁷. Since there are some limitations for sampling of the hairs in regard to each race, we used the commercial samples.

UV irradiation

UV irradiation was performed using a phototherapy system composed of six perpendicular UVA lamps (20 W, FL20SBLB, Sankyo Co., Ltd., Tokyo, Japan) and UVB (8 W, G8TE, Sankyo) (Fig. 1). Damaged hair samples were prepared at 12 and 48 hours after UVA (20 J for 12 hours and 40 J for 24 hours) and UVB (8 J for 12 hours and 16 J for 24 hours) irradiation. The distance from the lamp to the hair samples was 50 cm, and the relative humidity was 30%. A cooling fan was used to prevent the heat damage due to UV irradiation. All experiments were conducted at room temperature⁷.

Ultrastructural findings

1) Scanning electron microscopy (SEM)

Each prepared hair sample was affixed to a specimen slide, and gold coating was applied to the fixed hair sample with a sputter coating machine. Slides were examined by SEM (LEO 1499AP, 30 kV, LEO, Germany).

Results were scored according to a SEM standard grading system, as previously described by Kim et al.⁷.

2) Transmission electron microscopy (TEM)

All hair samples were incubated in 1 : 1 mixture of propylene oxide and epon overnight and then embedded in an epon mixture. Horizontal sections of approximately 60~70 nm were prepared and stained with uranyl acetate and lead citrate. The specimens were viewed under TEM (JEM-1200EDXII, 80 kV, JEOL, Tokyo, Japan)⁸. The results were scored according to the TEM standard grading system⁷.

3) Lipid transmission electron microscopic examination

The hair samples were fixed in Karnovsky solution (2% glutaraldehyde+2% paraform) and rinsed in 0.1 M sodium cacodylate and post-fixed with Lee's fixative (0.5% RuO₄ : 2% OsO₄ : 0.2 M cacodylate buffer = 1 : 1 : 1) to minimize hair injury^{1,7}.

Integral hair lipid analysis

Twenty milligrams of each hair sample were cut into 4 cm lengths, and continuously immersed in 5 ml of chloroform-methanol (2 : 1, 1 : 1, and 1 : 2) for 24 hours. The last immersion was in a chloroform-methanol-water (18 : 9 : 1) solution for 24 hours. The extracted solutions were pooled and filtered through a 0.5 μm Millipore filter. The filtrates were united and dried under a nitrogen stream, and the residue was recovered by dissolving the filtrates in 100 ul of the 1 : 1 chloroform-methanol mixture for further HP-TLC analysis. The integral hair lipids were dissolved in 100 ul of the 1 : 1 chloroform-methanol mixture, and 2 ul of each solution containing lipids were spotted onto silica gel-coated rods using a microdispenser.

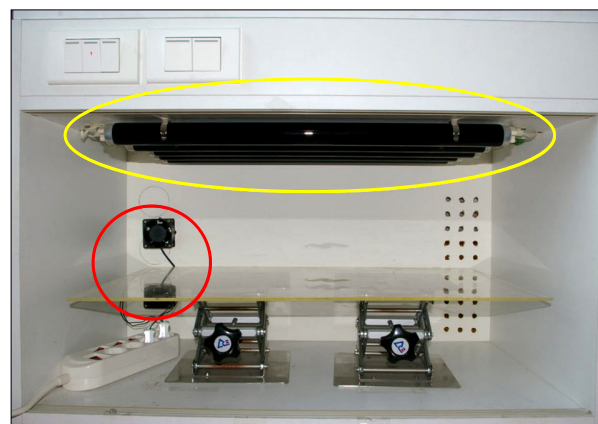


Fig. 1. The ultraviolet (UV) irradiator manufactured by the authors. UV light was controlled using the switch on the left upper side (yellow circle: UV lamps, red circle: cooling fan).

The rods were developed three times consecutively using the following: (i) hexane-diethyl ether-acetic acid (200 : 80 : 1) up to 6 cm; (ii) hexane-benzene (1 : 1) up to 8 cm; and (iii) hexane up to 10 cm. After each development, the rods were heated at 60°C for 5 minutes in order to dry. After completion, the rods were treated with diluted H₂SO₄, and were heated at 180°C for approximately 15 minutes.

RESULTS

Evaluation of hair surface damage by SEM

We evaluated the damage to the hair surface due to UV irradiation, using a standard grading system for damaged hair⁷. The hair cuticles from all three groups had intact and tightly overlapping cuticle scales. As the irradiation energy increased, the surfaces of samples from all three groups became increasingly damaged. Focal lift, loss of the cuticle edge, and exposure of the cortical cells were

observed in all samples. When the grading system was applied, the grading scores for each group increased as the exposure to UV irradiation increased (Table 1). UVB irradiation resulted in more severe damage than UVA irradiation, and compared to the samples from other groups, the African hair exhibited relatively worse damage (Fig. 2).

Evaluation of cuticle layer damage by TEM

All hair samples across groups had intact cuticles of greater than six layers. As the irradiation energy increased, variably sized holes, cleavage along the endocuticles, and cuticular detachment were observed in all groups. Consistent with the SEM results, the grading score increased with increased UV irradiation exposure (Table 2). UVA irradiation and UVB irradiation resulted in similar damage. The hair samples of all three groups exhibited similar patterns of damage. However, the African hair exhibited the weakest resistance to UV irradiation (Fig. 3).

Table 1. Grades of surface damage according to SEM

	Control	UVA 12 hr	UVA 48 hr	UVB 12 hr	UVB 48 hr
Asian	Scu0	Scu0	Scu2	Scu1	Scu2
European	Scu0	Scu0	Scu2	Scu1	Scu2
African	Scu0	Scu1	Scu2	Scu2	Scu2

Grades of the hair surface damage by SEM (Scu). Scu0: Virgin, intact hair, regular overlay of the cuticle. Scu1: Irregular overlay of the cuticle: crack (-), hole (-). Scu2: Severe lift-up of the cuticle, Crack (+) or hole (+), Cortex (-). Scu3: Cortex (partial +): Cuticle (+). Scu4: Cortex (+), Cuticle (-). SEM: scanning electron microscopy, UV: ultraviolet.

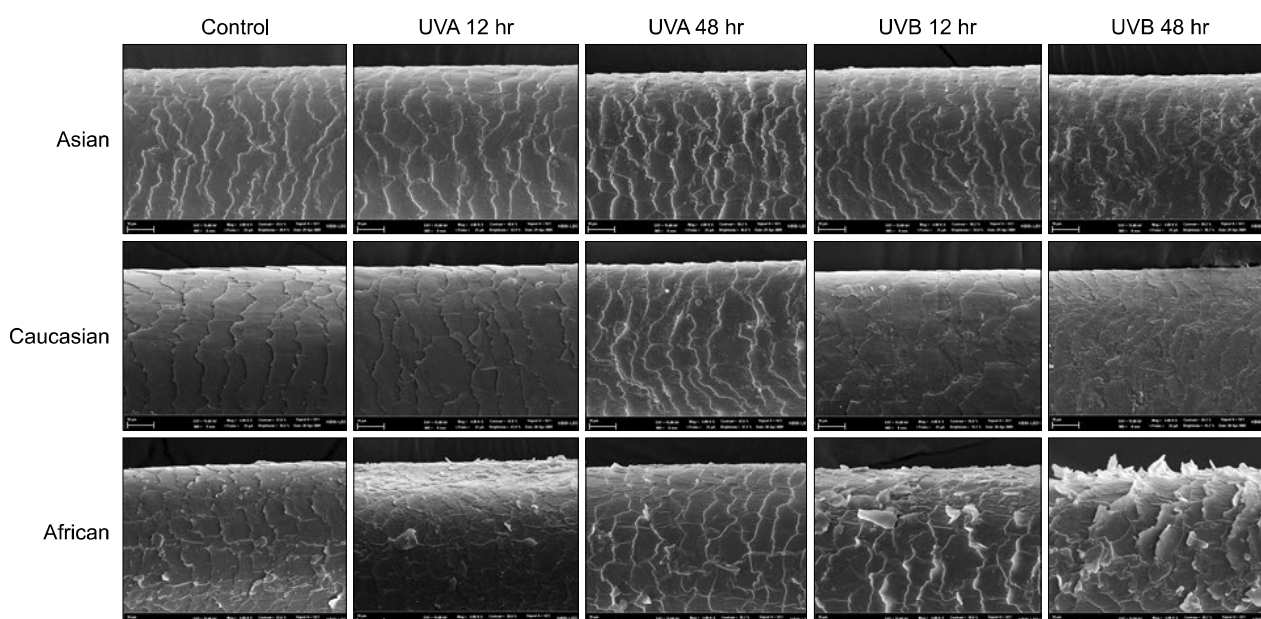


Fig. 2. Scanning electron microscopy findings for all hair samples. The greater the irradiation time, the greater the damage to the hair surface. Ultraviolet B (UVB) radiation resulted in more damage than UVA, and African hair showed the most damage to the hair surface.

Table 2. Grades of cuticle layer damage assessed by TEM

	Control	UVA 12 hr	UVA 48 hr	UVB 12 hr	UVB 48 hr
Asian	Scu0	Scu0	Scu1	Scu1	Scu1
European	Scu0	Scu1	Scu1	Scu2	Scu3
African	Scu0	Scu1	Scu2	Scu2	Scu4

Grades of the hair cuticle layers damage by TEM (Tcu). Tcu0: Intact cuticle layer, cuticle layers ≥ 6 layers. Tcu1: Outer cuticle detachment, Cuticle ≥ 5 layers. Tcu2: Bubbles in the endocuticle (+), Cuticle ≥ 5 layers. Tcu3: Remaining cuticle: 3~4 layers. Tcu4: Remaining cuticle ≤ 2 layers. TEM: transmission electron microscopy, UV: ultraviolet.

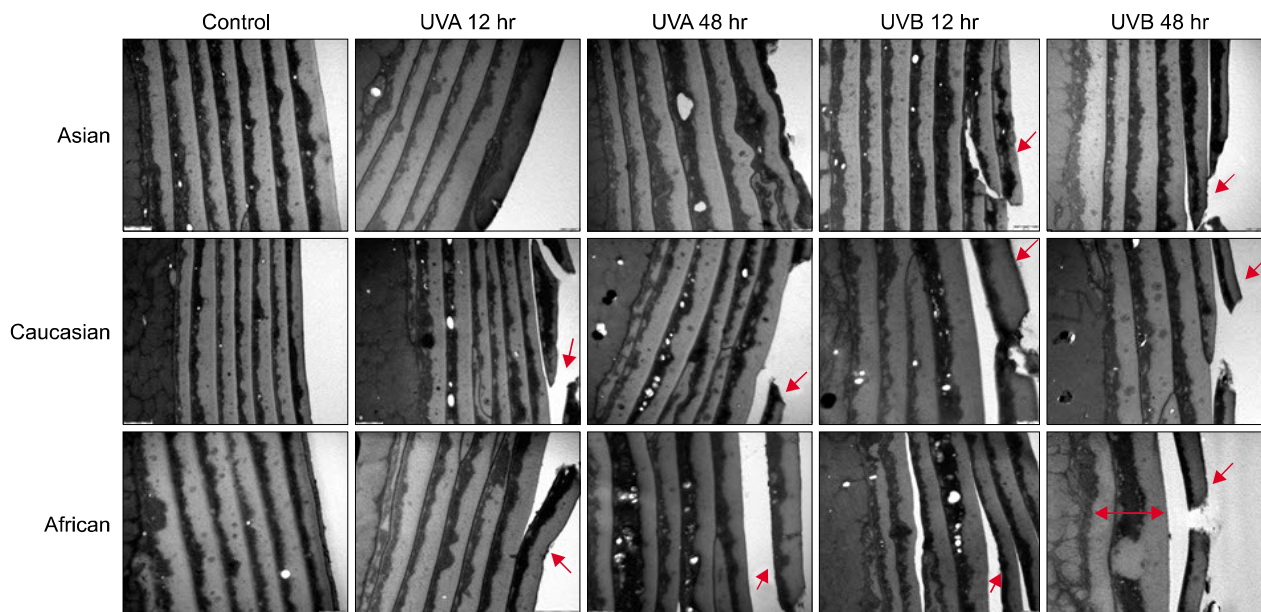


Fig. 3. Conventional transmission electron microscopy findings for all hair samples. Ultraviolet B (UVB) irradiation resulted in more damage than UVA irradiation (red arrow). All three groups exhibited similar patterns of damage. However, the African hair exhibited weaker resistance to UV irradiation than the other groups, and the African hair also exhibited a decreased number of cuticle layers than the other groups (red two-way arrow).

Evaluation of lipid layer damage by lipid TEM

All hair samples had intact intercellular lipid layers prior to UV irradiation. Following UV irradiation, we observed some focal bulging and disruption in the intercellular lipid layers. UVA and UVB irradiation resulted in similar changes to the intercellular lipid layers. UVA irradiation resulted in swelling of the lipid layer, and UVB irradiation caused disruptions to the lipid layer. We confirmed that the more energy exposure to UV radiation, the greater the damage to the lipid layer. In particular, African hair exhibited relatively weaker resistance to the UV irradiation than the samples from other groups (Fig. 4).

Integral hair lipid analysis by HP-TLC

Among the samples of the three groups, differences were found in the integral hair lipids. Asian samples had more integral hair lipids than others prior to UV radiation

exposure (Fig. 5A) and after exposure (Fig. 5B). Free fatty acid contents were higher than other lipids. Squalene content was higher in the African samples than in the other groups. UV irradiation decreased the lipid content, especially the free fatty acid content in the European and African samples. In the European and African samples, the levels of free fatty acids decreased when UV irradiation was performed. However, the Asian samples showed no change (Fig. 5).

DISCUSSION

The curliness, color, and cross-sectional parameters of hair samples differ across human ethnic groups. However, some proteins, keratins, and molecular structures, including amino acids, have common features across groups⁹⁻¹¹. Although there are no significant geographical differences in keratin proteins, which are the main structural components

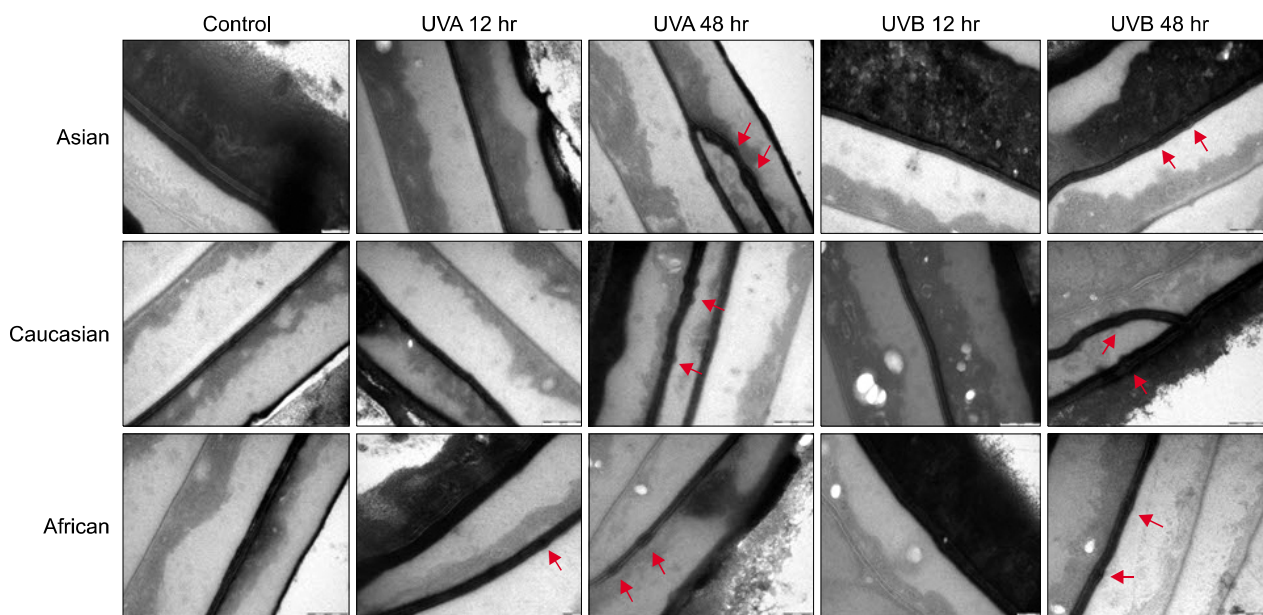


Fig. 4. Lipid transmission electron microscopy findings for all hair samples. Ultraviolet (UV) irradiation resulted in swelling of the lipid layer (red arrow). However, there were no differences observed among groups after UV irradiation.

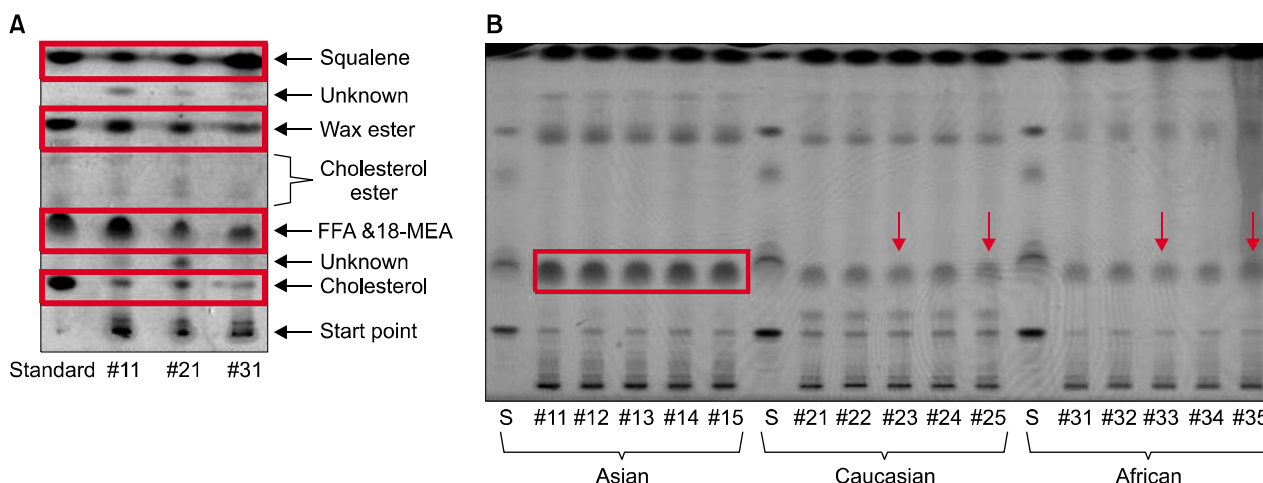


Fig. 5. High-performance thin-layer chromatography (HP-TLC) analysis of ultraviolet (UV) light irradiated hair samples. The Asian hair had more integral hair lipids than the other hairs. In particular, the free fatty acid content was higher than those of the other lipids. UV irradiation resulted in decreased lipid content, especially free fatty acids. (A) Amount of integral hair lipids between ethnic groups. (B) Integral hair lipids after UV radiation. S, standard; Control, #11, #21, #31; UVA 12 hr, #12, #22, #32; UVA 48 hr, #13, #23, #33; UVB 12 hr, #14, #24, #34; UVB 48 hr, #15, #25, #35.

of hair, physical characteristics differ among groups. Other components, such as melanin and lipid components, affect the characteristics of hair. Franbourg et al.⁹ reported that African hair exhibited less radial swelling when flushed with water compared to Asian or European hair and the authors assumed that there might be differences in hair lipids among human populations. However, proper lipid analysis of this study was not performed. Additionally, Syed et al.¹² reported differences in moisturizing rates

associated with the differences in lipid content. In hair care, many experts recognize the importance of moisturization; however, it is difficult to observe integral hair lipids and distinguish them from the lipids produced by sebaceous glands.

The lipid layer is important for maintaining hair integrity; thus, it is also referred to as the "hair barrier". During the process of keratinization in the cortex, an adhesive layer (CMC) forms between adjacent cells through the cortical

plasma membrane. CMC is a continuous structure composed of three-layered structures, and the middle layer (δ -layer) is surrounded by inner and outer β -layers. The integral hair lipid is located in the beta-layer¹³. The lipid layer of hair is composed mainly of fatty acids, cholesterol sulfate, ceramide, and cholesterol. Orwin¹⁴ observed that globular particles in the intercellular spaces form lamellar structures during hair development. When they migrate to the external area and membrane rupture is occurred, these particles form the lamellar structure. Finally, a lipid layer builds up between the outside of the hair cuticle and the IRS cuticle. One of the most important lipid components is 18-methyleicosanoic acid (18-MEA), which occupies roughly 40% of all fatty acids and has an ester bond or thioester bond with the keratinized cell¹⁵. CMC is a major component of 18-MEA, and it is the lipid layer that decreases friction and increases hydrophobicity of the hair surface¹³. We determined that samples from Asians demonstrate higher levels of integral hair lipids than other groups. Free fatty acids, cholesterols, and wax esters were also higher in Asian samples. African samples exhibited higher levels of squalene, but overall, lipid content was lower for this group than for Europeans or Asians. Previous reports^{9,12} using hair swelling revealed that hair lipids of Africans may be more prominent than other ethnic groups. Both our results and previous results may be contradictory to one another. Previously reported results were made from non-standardized hair samples and it may cause the cause for the different results. We used standardized hair samples but there are many factors which can affect the lipid contents that cannot be determined. To analyze more accurate lipid contents, further advanced studies are needed.

UV light is composed of UVA (320~400 nm), UVB (290~320 nm), and UVC (200~290 nm) waves. UVC is blocked by the ozone layer, but UVA and UVB affect skin and hair. Damage to hair due to UV light occurs due to free radicals or cysteic acid, which forms after UV radiation and breaks disulfide bonds^{16,17}.

In this study, SEM and TEM analyses showed similar patterns of hair damage, regardless of population origin. As UV irradiation increased, greater focal lifting and loss of the cuticle edge was observed. Furthermore, the number of cuticle layers decreased after 48 hours of UV irradiation. We confirmed that hair surfaces are damaged primarily by UVB light, while hair lipids are damaged primarily by UVA light. These results may be due to differences in the penetration depth of UV light⁴.

Damage to the integral lipid layer was similar across groups. Normal hair had a uniform lipid layer, but UV damaged hair displayed swelling of the lipid layer and

disruption. The bulging of the lipid layer was mainly observed in samples exposed to UVA irradiation, and the disruption of the continual lipid layer was mainly observed in samples exposed to UVB irradiation.

Lipid content was higher in Asian samples than in other groups, while free fatty acid levels were lower in African samples than in other groups. After UV irradiation, the Asian samples were less damaged than the African samples, while the damage to the European and African samples was time dependent. Thus, we inferred that the quantity of free fatty acid is likely associated with hair damage though there are several additional factors that might also account for the observed effect, such as fiber geometry, diameter and pigment content which need to be investigated from this point forward.

In conclusion, we compared and analyzed the integral hair lipids across three populations and observed morphological changes of hair lipids following UV irradiation. We determined that Asian hair contains more integral hair lipids than European or African hair. After UV irradiation, African and European hair samples exhibited more damage than Asian samples. Therefore, we concluded that integral hair lipids may protect hair against UV light.

ACKNOWLEDGMENT

This work was supported by a research grant from Yonsei University Wonju College of Medicine (YUWCM 2011-63).

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