pISSN 1226-2587
J. Soc. Cosmet. Sci. Korea eISSN 2288-9507
Vol. 41, No. 4, December 2015, 413-420 http://dx.doi.org/10.15230/SCSK.2015.41.4.413

한국 여성의 피부 부위 및 연령에 따른 피부 측정 인자와 생물 인자 연구

조 석 철·남 개 원*†

서원대학교 식품공학과, *서원대학교 화장품과학과, 서원 글로벌 피부임상센터
(2015년 10월 20일 접수, 2015년 11월 15일 수정, 2015년 12월 10일 채택)

A Study of Skin Biophysical Parameters and Biomarkers related to the Anatomical Site and Age in Korean Women

Seok-Cheol Cho and Gaewon Nam*†

Department of Food Engineering, Seowon University, 377-3 Musimseoro, Seowon-gu, Cheongju, Chungbuk 28674, Korea
*Department of Cosmetic Science & Technology, Seowon University, Seowon Global Skin Research Center, Chungbuk 28674, Korea
(Received October 20, 2015; Revised November 15, 2015; Accepted December 10, 2015)

요 약: 피부는 신체에서 가장 크고 육중한 기관 중 하나로 인간의 생리 및 병리 과정에 참여한다. 피부는 자기 유지 및 치유, 기계적 및 화학적 손상 방어, 자외선 과 외부 병원성 미생물로부터의 방어, 비타민 D 합성 그리고 사회 심리적 기능을 한다고 알려져 있다. 이 연구의 목적은 한국 여성의 부위와 연령에 따라 피부 생물학 인자와 연관된 생물리학 인자의 변화를 평가하는 데 있다. 20 ~ 49세의 약 70명의 건강한 성인 여성이 이 실험에 참여하였다. 측정부위는 하박 내측과 뺨으로 진행하였다. 인체 피부의 생물리학 인자를 측정하기 위하여 여러 가지 비침습적인 방법으로 진행하였다. 피부의 생물학 인자를 분석하기 위하여 코티졸, 파이브로넥틴, 케라틴−1, 10, 11, 인보루크린, 케라틴 6를 인체의 얼굴과 하박내측으로 비교하였다. 또한 비침습적 방법으로 피부 생물리학 인자는 피부 부위와 연령에 따른 차이를 측정하였다. 측정 부위에 따른 결과, 각질층 수분량, 경피수분손실량과 피부색(L과 a값)은 유의적인 차이가 나타났다. 연령에 따른 결과, 오직 피부색에서만 연령에 따른 차이가 유의적으로 나타났다. 코티졸, 케라틴−6, 파이브로넥틴, 케라틴−1, 10, 11 은 연령과 부위간 유의적 차이가 없지만 인볼루크린은 30 ~ 39세 연령대에서 다른 연령대보다 유의적으로 가장 높았다. 이러한 결과는 개인의 피부 환경에 대한 상세한 피부 상태 변화로 설명할 수 있을 것이다.

Abstract: The skin is one of the largest organs in our body and participates in many of the human organism's physiological and pathological events. Skin function were known for self-maintenance and self-repair, mechanical and chemical stress protection, protection against UV and environmental pathogenic micro-organisms, production of vitamin D, and social and psychological function through the physical aspect. The aim of this study was to evaluate the variation of biophysical parameters and to find relation with skin biomarkers in different anatomical site and age in Korean women. About 70 healthy volunteers in age range 20 to 49 were participated in this test. Test areas were the forearms and the cheek. Investigation to determine biophysical parameters on human skin, was carried out using various non-invasive methods. For analysis to skin biomarkers, we studied to examine various biomarkers for the quantitative determination of cortisol, fibronectin, keratin-1, 10, and 11, involucrin, and keratin-6 in human face and forearm. And we measured to skin biophysical parameters for skin anatomical site and age difference with non-invasive methods. As results of measuring site, some parameters were have following significant difference, stratum corneum hydration, trans epidermal

† 주 저자(e-mail: skarod@seowon.ac.kr)
call: 043)299-8496

water loss and skin color (L and a value). As results of age difference, skin colors were had only significant difference with age. For cortisol, keratin-6, fibronectin, keratin-1, 10, 11 contents, there were no significant difference in age and site. However, involucrin level in the cheeks were the highest for age group 30 ~ 39 compared to other age groups. These results suggest that in individual skin condition may explain detailed skin state variation.

Keywords: *Non-invasive measurement, biomarker, age and anatomical site*

1. Introduction

Intrinsic and extrinsic aging are two primary skin aging processes. Variations in individual genetic background play a role in the intrinsic aging. Therefore, intrinsic aging is not preventable and cannot be controlled. However, extrinsic aging is due to external factors such as smoking, excessive alcohol consumption, poor nutrition, and chronic UV exposure. These factors contribute to premature aging. The most significant and harmful contribution to extrinsic aging is chronic UV exposure, which contributes about 80% to premature aging[1].

Intrinsic aging is characterized by smooth, unblemished skin with some wrinkles. There is in an increase in the collagen fibrils[2]. In addition, epidermal and dermal atrophy along with decrease in fibroblasts and mast cells can be seen[3-4]. There is in an increase in the collagen fibrils. At the cellular level for intrinsic aging, telomeres, which are specialized structures found at the ends of eukaryotic chromosomes, are known to play an important role in protecting the end of the chromosome from deterioration or from fusion with neighboring chromosomes. Intact telomeres extend the lifespan of cells. The length of telomere shortens with age. Therefore, shortening of telomere is one of the measuring factors for intrinsic aging.

Face, chest, arms, and legs are exposed to the UV more than any other parts of the body. In photo-aged skin, rhytides, pigmented lesions, such as patchy hyperpigmentation, and depigmented lesions, such as guttate hypomelanosis, are present. In addition, there is a loss in the tone and elasticity along with an increase in the skin fragility. In photoaged skin, there is epidermal atrophy along with distinct alterations in collagen and elastic fibers. In cases where there has been severe photoaging, fragmented and thickened collagen fibers are seen[5]. In addition, there is fragmentation of elastic fibers along with progressive cross-linkage and calcification[6]. Further exposure to the UV radiation increases in the collagen and elastic fibers' deterioration.

As skin ages, about 20% of the dermal thickness disappears[6]. There are changes in the collagen production and photo-aged dermis is characterized by disorganized collagen fibrils with accumulation of abnormal elastin containing material[6-7]. However, in the aged skin, the ratio of the Type III to Type I collagen increases. This is due to lose in the collagen I[8]. Also, with increase in age, the overall collagen content per unit area the skin surface is known to decline about 1% / year[9]. In photo-aged skin, amount of the collagen I is reduced to about 59%[6, 10].

UVR exposure up-regulates the synthesis of several types of collagen degrading enzymes known as the matrix metalloproteinases (MMPs). First, the transcription factor, c-Jun, is increased with UV exposure. c-Fos, with is another transcription factor present in high amount even without sun exposure, is also involved with MMPs. Activator protein-1 (AP-1) is formed by combination of the c-Jun and c-Fos. Then, AP-1 activates the MMP genes, which stimulates the production of collagenase. Collagenase attacks and degrades collagen. Increase in the amount of formation in the collagenase results in degradation of collagen[6].

In photo-aged skin, alterations in the elastic fibers are significant. Thickening and coiling of elastic fibers in the papillary dermis occurs in the photo-aged skin. These changes also occur in the reticular dermis as a result of chronic UV exposure[6]. Initially, in photo-damage, the elastic fibers amount increases. The level of the sun exposure determines the degree of the hyperplastic response[6,11].

Aged skin is often characterized by dry, scaly skin. This is due to degradation or loss of the skin barrier function. Since the recovery from a damaged barrier function is slower in aged skin, elders are prone to developing dryness. In addition, in aged skin, there is an increase in the trans-epidermal water loss. This contributes to the dry skin especially in low humidity temperatures[6].

The aim of this study was to evaluate the variation of biophysical parameters and to find relation with skin biomarkers in different anatomical site and age in Korean women.

2. Materials and Methods

2.1. Volunteers and Environmental Condition

In total, 63 healthy female volunteers (aged 20 ~ 49, mean age 34.6 years) without any indication of cutaneous pathology participated in this study. All volunteers participating lived in Korea more than five years. Volunteers visited the controlled rooms, where the temperature and humidity (22 ± 2 ℃, 50 ± 5%) was controlled, and used general facial cleanser to wash the face and the forearm. Then, the volunteers wait for at least 30 min in the controlled room. Various non-invasive measurements were collected. Then, the stratum corneum samples were obtained. The data were collected between March and May. The study was previously approved by the ethical committee of university of Seowon (SWEC5-1-17).

2.2. Non-Invasive Measurements

Skin biophysical parameters were obtained by using various measuring systems. Epidermal permeability barrier function, stratum corneum hydration, skin color and skin surface pH information were collected. The epidermal permeability barrier function was evaluated using trans-epidermal water loss (TEWL) with a Vapometer® (Delfin, Finland), stratum corneum hydration was evaluated using electrical capacitance with a Corneometer® CM825 (C+K Electronic GmbH, Germany) and skin surface pH was collected using a pH meter PH900 (C+K Electronic GmbH, Germany)[12-14]. Skin colors were measured with a Chromameter CM2002 (Minolta, Japan)[15]. For assessing skin color, the Chromameter was employed in the $L^*a^*b^*$ system. The color is expressed in a three-dimensional space. The L^* value (luminance) gives the relative brightness, ranging from total black ($L^* = 0$) to total white ($L^* = 100$). The a^* value represents the color range from red (positive values) to green (negative values), whereas the b^* value is the range from color yellow to blue.

2.3. Skin Biomarker Assay

Stratum corneum samples were obtained by tape stripping using D-squame® (Cuderm, USA) from the cheek and the forearm of healthy Korean women. Informed consent was obtained prior to the experiments. For the cheek, cheek was washed 30 min. prior to tape stripping. These samples were then extracted into buffer for analysis. Utilizing the Luminex-based bead arrays, multiple analyte profiling kit was used for the quantitative determination of cortisol, fibronectin, keratin-1, 10, and 11, involucrin, and keratin-6[16-17]. Each sample is examined using a Lincoplex® SkinMAP system in duplicate.

2.4. Statistical analysis

Statistical analysis was performed using the SPSS (Statistical Package for the Social Science) 20.0 software (IBM SPSS, USA). All data are expressed as mean ± SE (Standard Error) in tables and figures. Statistical difference of skin parameters between the sites were calculated by paired *t*-test and statistical difference of age group were also calculated by independent *t*-test, significance level ($p < 0.05$)[18].

3. Results and Discussion

3.1. Skin Hydration

We investigated the difference of age and anatomical site in Korean women. Skin biophysical parameters revealed significant site and age difference. The effect of skin hydration was shown Table 1 and Figure 1A. Hydration level (a.u.) in the cheek for women in 20's,

Table 1. Comparison of the Age Related Skin Properties: Biophysical Parameters and Biomarkers

Site	Age groups	Fibronectin	Cortisol	Keratin-6	Keratin-1,10,11	Involucrin	Hydration	TEWL	Skin pH	L*	a*	b*
Cheek	20's	0.12 ± 0.05	7.76 ± 0.88	2.33 ± 0.24	39.31 ± 3.04	0.043 ± 0.01	43.17 ± 2.04	17.63 ± 0.91	5.52 ± 0.07	65.34 ± 0.26	22.07 ± 0.64	19.19 ± 0.35
	30's	0.07 ± 0.01	6.97 ± 1.10	3.10 ± 0.54	40.19 ± 2.98	0.139 ± 0.04	41.79 ± 2.53	20.36 ± 1.41	5.47 ± 0.05	64.03 ± 0.41	21.88 ± 0.44	20.59 ± 0.43
	40's	0.09 ± 0.03	8.53 ± 1.04	2.73 ± 0.63	38.44 ± 2.65	0.035 ± 0.01	41.88 ± 2.13	19.05 ± 1.83	5.44 ± 0.10	63.65 ± 1.01	21.23 ± 0.68	19.89 ± 0.27
Forearm	20's	0.07 ± 0.01	7.34 ± 1.00	2.44 ± 0.35	42.56 ± 3.38	0.004 ± 0.00	26.67 ± 1.04	5.93 ± 0.38	5.43 ± 0.08	69.10 ± 0.45	12.92 ± 0.22	18.28 ± 0.37
	30's	0.07 ± 0.02	7.40 ± 1.10	2.38 ± 0.18	36.76 ± 2.71	0.006 ± 0.00	27.09 ± 1.23	7.11 ± 0.54	5.38 ± 0.10	67.17 ± 0.32	14.62 ± 0.25	20.79 ± 0.35
	40's	0.08 ± 0.01	8.24 ± 1.08	2.09 ± 0.00	41.84 ± 3.52	0.005 ± 0.00	27.47 ± 1.44	5.81 ± 0.47	5.53 ± 0.14	67.92 ± 0.66	14.00 ± 0.45	19.87 ± 0.42

Data Expressed in Mean ± SE (Standard Error), 20's n = 24 (Aged 25.3 ± 2.6), 30's n = 22 (Aged 36.4 ± 2.5), 40's n = 17 (Aged 23.9 ± 2.9)

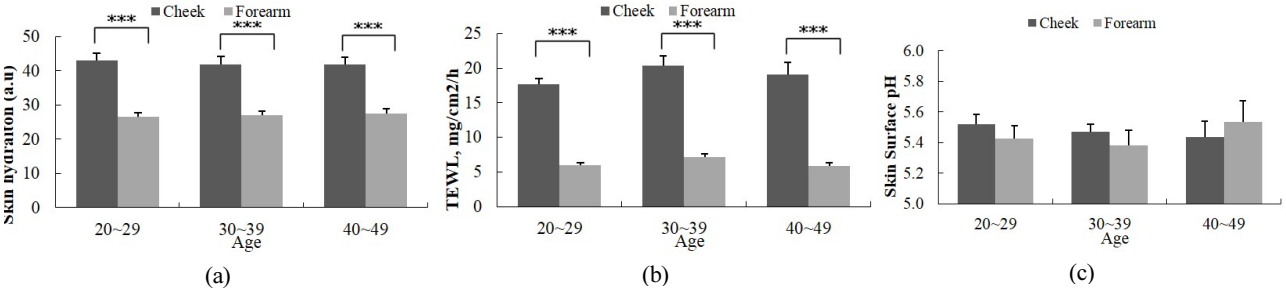


Figure 1. Age related variation of (a) skin hydration, (b) barrier function, and (c) skin surface pH. Site and age group *t*-test ($^{***}p < 0.001$).

30's, and 40's were 43.17, 41.79, and 41.88, respectively. The skin hydration values were significantly higher in the cheek than in the forearm. But age related results showed no significant difference ($p > 0.05$).

3.2. Skin Barrier Function

The epidermal permeability barrier functions (TEWL, tans epidermal water loss) were obtained similar results (Figure 1B). The TEWL in the cheek for women in 20's, 30's, and 40's were 17.63, 20.36, and 19.05, respectively. The TEWL in the forearm for women in 20's, 30's, and 40's were 5.93, 7.11, and 5.81, respectively.

3.3. Skin Surface pH

As shown Figure 1C, pH in the cheek for women in 20's, 30's, and 40's were 5.52, 5.47, and 5.40, respectively. However, the pH in the forearm for women in 20's, 30's, and 40's were 5.43, 5.38, and 5.53, respectively. The variation of skin surface pH values were no significant difference as age and site variation.

3.4. Skin Brightness (L*)

The skin brightness in the cheek for women in 20's, 30's, and 40's were 65.34, 64.03, and 63.65, respectively. Skin brightness in the forearm, however, was 69.10, 67.17, and 67.97 for women in 20's, 30's, and 40's, re-

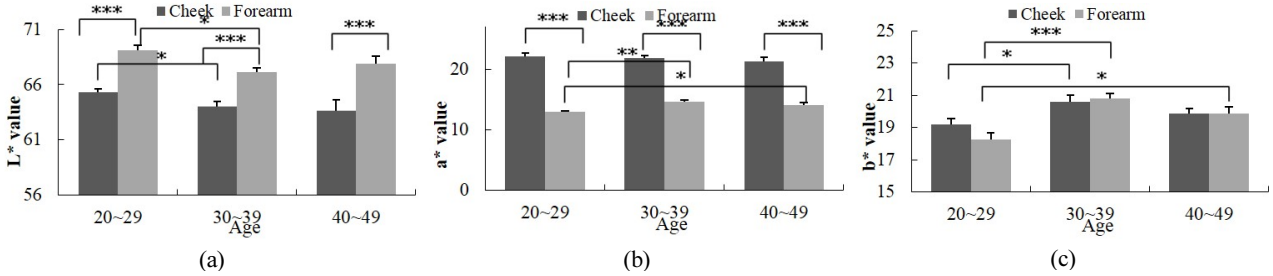


Figure 2. Age related variation of (L^*, (a)) skin brightness, (a^*, (b)) redness, and (b^*, (c)) yellowness. Site and age group *t*-test ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

spectively (Figure 2A). The forearm color was brighter than the cheek in all age groups ($p < 0.05$). Interestingly, higher age groups, the L^* value was tend to decrease in the cheek. In the cheek, there was a correlation between women in 20's and 30's however not in 20's and 40's.

3.5. Skin Redness (a^*)

Skin redness (a^*) in the cheek was 22.07, 21.88, and 21.23 for women in 20's, 30's and 40's, respectively. However, for the forearm, the results were 12.92, 14.62, and 14.00 for women in 20's, 30's and 40's, respectively. The a^* value was significant higher in the cheek than in the forearm (Figure 2B). The a^* value represents color range from red to green in the skin, the cheek was more red color than the forearm.

3.6. Skin Yellowness (b^*)

Skin yellowness (b^*) in the cheek was 19.19, 20.59, and 19.89 for women in 20's 30's and 40's, respectively. However, in the forearm, the results were 18.28, 20.79, and 19.87 for women in 20's, 30's and 40's, respectively. Site of measurement did not impact on the results for skin yellowness. The b^* value was significant higher in 30 ~ 39 and 40 ~ 49 age group than in 20 ~ 29 age group (Figure 2C). Over the 30 age, the b^* value was tend to increase in the cheek and forearm.

3.7. Skin Biomarkers

The most skin biomarkers were not significant difference in age and site variation. But the involucrin contents were significant difference in age and site (Figure 3A).

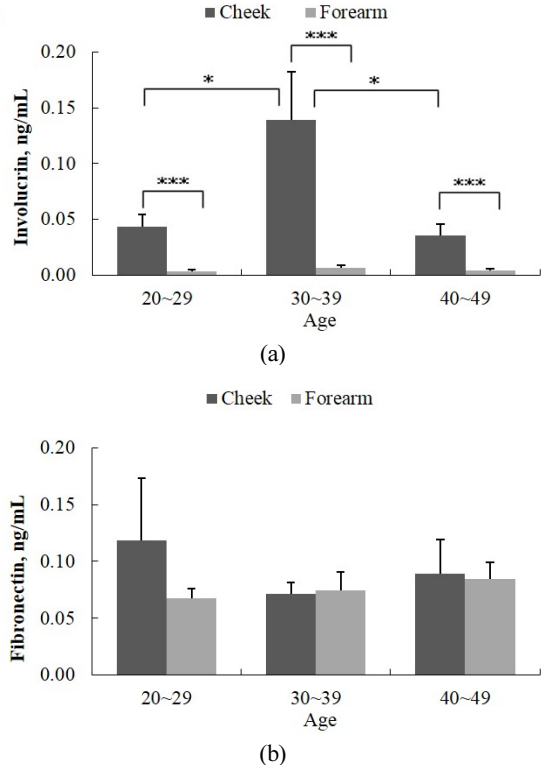
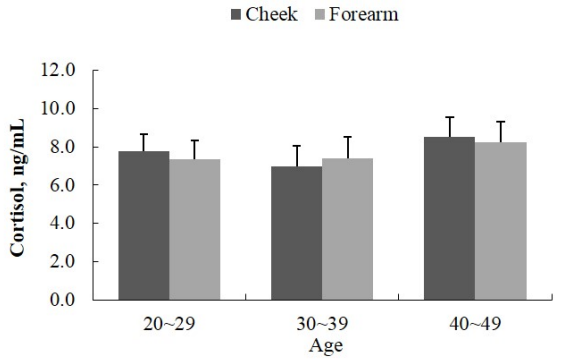
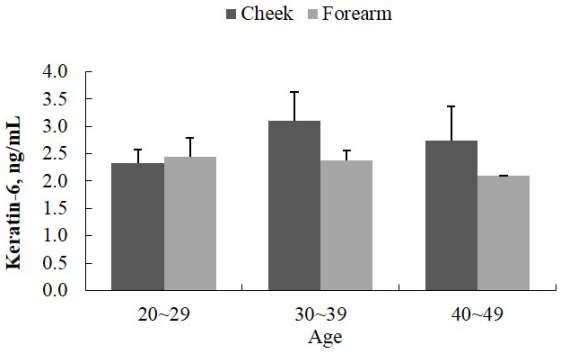


Figure 3. Age related variation of (a) involucrin and (b) fibronectin. Site and age group. *t*-test ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Involucrin level for women in 20's, 30's and 40's were 0.043 ng/mL, 0.139 ng/mL, and 0.035 ng/mL, respectively, in the cheek. In the forearm, the results were reported to be as 0.004 ng/mL, 0.006 ng/mL, and 0.005 ng/mL for the same age group. Another biomarker, Fibronectin level in the cheek for women in 20's, 30's, and 40's were 0.12 ng/mL, 0.07 ng/mL, and 0.09 ng/mL, respectively (Figure 3B). Fibronectin level in the forearm



(a)



(b)

Figure 4. Age related variation of (a) cortisol and (b) keratin-6. Site and age group. *t*-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

for women in 20's, 30's, and 40's were 0.07 ng/mL, 0.07 ng/mL, and 0.08 ng/mL, respectively.

Cortisol level in the cheek for women in 20's, 30's, and 40's were 7.76 ng/mL, 6.97 ng/mL, and 8.53 ng/mL, respectively (Figure 4A). Cortisol level in the forearm for women in 20's, 30's, and 40's were 7.34 ng/mL, 7.40 ng/mL, and 8.24 ng/mL, respectively. Keratin-6 level in the cheek for women in 20's, 30's, and 40's were 2.33 ng/mL, 3.10 ng/mL, and 2.73 ng/mL, respectively (Figure 4B). Keratin-6 level in the forearm for women in 20's, 30's, and 40's were 2.44 ng/mL, 2.38 ng/mL, and 2.09 ng/mL, respectively.

Keratin-1, 10, 11 in the cheek for women in 20's, 30's, and 40's were 39.1 ng/mL, 40.19 ng/mL, and 38.44 ng/mL, respectively (Figure 5). Results in the forearm were 42.56 ng/mL, 36.76 ng/mL, and 41.84 ng/mL for women in 20's, 30's and 40's, respectively.

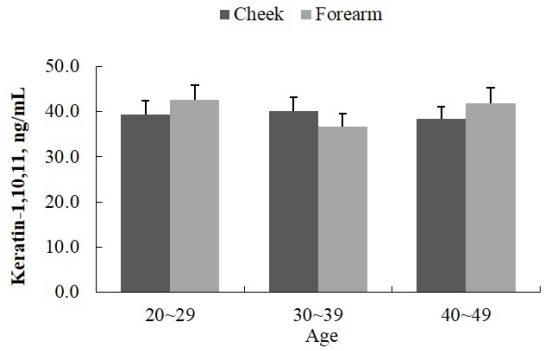


Figure 5. Age related variation of keratin-1, 10, 11 between cheek and forearm and among age groups. *t*-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Fibronectin, cortisol, keratin-6, and keratin-1, 10, 11 were have no significant difference in age and site (Figure 3B, 4, 5, Table 1).

3.8. Discussion

As aging process occurs, there is an increase in the wrinkle and decrease in the elasticity. Qui *et al*.[19] reported that certain skin properties are specific for certain ethnic groups. Crow's feet wrinkle, underneath eye wrinkles, and forehead wrinkles increase with age. In addition, skin brightness decreases, sebum excretion decreases, and the pigmented spot increases[19]. Melanin index increases while the hydration level decrease. This occurs more in the summer than in the winter.

In this study, as with increasing age, skin brightness (L^{*}) in the cheek was decrease. Women in the 30's, both in the cheek and in the forearm, and forearm of women in the 40's, had higher significant value for skin yellowness compared to women in the 20's. Glycation and carbonylation in the aging skin is responsible for the skin yellowness[20]. Hydration level in the 20's was higher than in 30's or 40's. However, there is no specific relationship between increased in the hydration level with increasing age. Furthermore, there is no specific relationship between changes in the TEWL or pH with age. However, this study only included subject from the age group ranging from 20's to 40's. Therefore, results from this study can be different from other studies, where the subjects'

age ranged from ten to seventy.

Biomarkers were measured from the corneocytes samples. Fibronectin is a high-molecular weight (~ 440 kDa) glycoprotein of the extracellular matrix that binds to membrane-spanning receptor proteins called integrins. Cortisol is produced by the adrenal gland, which are located just above the kidney. It is a stress hormone. Therefore, it is released in response to stress and a low level of blood glucocorticoids. Its primary functions are to increase the blood sugar through gluconeogenesis. However, in the skin, cortisol is known to slow the skin's recovery system. Kerain-6 is related to cellular proliferation. However, it has an abnormal differentiation. However, keratin-1, 10, 11 is related to epidermal differentiation. It is a cytokine result of early differentiation. The involucrin contents were represented the epidermal differentiation marker and were known to expressed the immature corneocytes. In binding the protein loricrin, involucrin contributes to the formation of a cell envelope that protects corneocytes in the skin.

Studying and analyzing the stratum corneum in this study, shows that there may not be a relationship between biomarker level and age group. However, there was a high level of involucrin level for women in their 30's. It have been not explained why 30 ~ 39 age group were expressed the highest involucrin contents. Further studies are necessary to clarify the mechanisms of detect of involucrin contents in skin disorders.

High level of involucrin in the 30's can refer that this age group has a high formation of corneocytes envelope compared to other age groups. There is a lipid barrier and above this barrier there is corneocytes barrier. In a small scale, high level of involucrin can refer that there is solid, inflexible keratinocytes. In a large scale, it can refer that the stratum corneum layer is rigid and solid. However, since the skin is exposed to the external environment, involucrin level may be high due to exposure to the sun[21]. Further lifestyle research should be conducted to answer the questions related to high level of involucrin in the 30's.

4. Conclusion

1) Skin hydration values and epidermal permeability barrier functions were significantly higher in the cheek than in the forearm on all age groups.
2) Skin surface pH values were no significant difference in age and site.
3) The forearm color (L^* values, skin brightness) was brighter than the cheek in all age groups.
4) Cortisol, keratin-6, fibronectin, keratin-1, 10, 11 contents were no significant difference in age and site.
5) Involucrin contents in the cheeks were had the highest in 30 ~ 39 age group than other age groups.

Reference

1. J. Uitto, Understanding premature skin aging, *N. Engl. J. Med*., **337**(20), 1463 (1997).
2. C. R. Lovell, K. A. Smolenski, V. C. Duance, N. D. Light, S. Young, and M. Dyson, Type I and III collagen content and fibre distribution in normal human skin during ageing, *Br. J. Dermatol*., **117**(4), 419 (1987).
3. N. A. Fenske and C. W. Lober, Structural and functional changes of normal aging skin, *J. Am. Acad. Dermatol*., **15**(4 Pt 1), 571 (1986).
4. G. Roupe, Skin of the aging human being, *Lakartidningen*, **98**(10), 1091 (2001).
5. R. M. Lavker, Structural alterations in exposed and unexposed aged skin, *J. Invest. Dermatol*., **73**(1), 59 (1979).
6. L. Baumann, Skin ageing and its treatment, *J. Pathol*., **211**(2), 241 (2007).
7. M. El-Domyati, S. Attia, F. Saleh, D. Brown, D. E. Birk, F. Gasparro, H. Ahmad, and J. Uitto, Intrinsic aging vs photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin, *Exp. Dermatol*., **11**(5), 398 (2002).
8. A. Oikarinen, The aging of skin: chronoaging versus photoaging, *Photodermatol. Photoimmunol. Photomed*.,

7(1), 3 (1990).
9. S. Shuster, M. M. Black, and E. McVitie, The influence of age and sex on skin thickness, skin collagen and density, *Br. J. Dermatol*., **93**(6), 639 (1975).
10. C. E. Griffiths, A. N. Russman, G. Majmudar, R. S. Singer, T. A. Hamilton, and J. J. Voorhees, Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid), *N. Engl. J. Med*., **329**(8), 530 (1993).
11. G. J. Fisher, S. Kang, J. Varani, Z. Bata-Csorgo, Y. Wan, S. Datta, and J. J. Voorhees, Mechanisms of photoaging and chronological skin aging, *Arch. Dermatol*., **138**(11), 1462 (2002).
12. D. Black, A. D. Pozo, J. M. Lagarde, and Y. Gall, Seasonal variability in the biophysical properties of stratum corneum from different anatomical sites, *Skin Res. Technol*., **6**(2), 70 (2000).
13. E. Berardesca, EEMCO guidance for the assessment of stratum corenum hydration: electrical methods, *Skin Res. Technol*., **3**(2), 126 (1997).
14. J. L. Parra and M. Paye, EEMCO guidance for the *in vivo* assessment of skin surface pH, *Skin Pharmacol. Appl. Skin Physiol*., **16**(3), 188 (2003).
15. G. E. Pierard, EEMCO guidance for the assessment of skin colour, *J. Eur. Acad. Dermatol. Venereol*., **10**(1), 1 (1998).
16. A. Pierre, Measuring the skin, Berlin, *Springer*, 173 (2004).
17. V. Terskikh, Y. A. Vorotelyak, and A. Vasiliev, Self-renewal of stem cells, *Acta. naturae*, **1**(2), 61 (2009).
18. T. Mauricio, Y. Karmon, and A. Khaiat, A randomized and placebo-controlled study to compare the skin-lightening efficacy and safety of lignin peroxidase cream vs 2% hydroquinone cream, *J. Cosmet. Dermatol*., **10**(4), 253 (2011).
19. H. Qiu, X. Long, J. C. Ye, J. J. Senee, A. Laurent, R. Bazin, F. Flament, A. Adam, J. Coutet, and B. Piot, Influence of season on some skin properties: winter vs summer, as experienced by 354 Shanghaiese women of various ages, *Int. J. Cosmet. Sci*., **33**(4), 377 (2011).
20. Y. Ogura, T. Kuwahara, M. Akiyama, S. Tajima, K. Hattori, K. Okamoto, S. Okawa, Y. Yamada, H. Tagami, M. Takahashi, and T. Hirao, Dermal carbonyl modification is related to the yellowish color change of photo-aged Japanese facial skin, *J. Dermatol. Sci*., **64**(1), 45 (2011).
21. M. Simon and H. Green, Involucrin in the epidermal cells of subprimates, *J. Invest. Dermatol*., **92**(5), 721 (1989).