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Noninvasive measurement of advanced glycation end-products in the facial skin: New data for skin aging studies

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Synopsis

Using skin autofluorescence (SAF) as a marker of advanced glycation end-products (AGEs) has been extensively studied in the last decade since the introduction of the noninvasive *in vivo* measurement technique. Data have shown the level of skin AGEs increases with chronological age in healthy human beings, and this increase is substantially higher in age-matched diabetic patients. In skin research, glycation with the accompanying accumulation of skin AGEs has been regarded as one of the primary skin aging mechanisms that contribute to skin wrinkling and the loss of skin elasticity. To date, the totality of SAF data reported in literature has been obtained from measurements on the arm, and noninvasive measurement of facial skin AGE accumulation would add great value to skin aging research. In this study, we report the levels of facial and forearm skin AGEs in 239 men and women of 21–65 year of age. Significantly lower levels of AGEs were detected in the facial skin than in the forearm skin from the young Caucasian groups, and the difference was much larger for men than for women. The rate of change in skin AGE level over age was found to be about 50% higher in men than in women, which further highlights the gender difference. A statistically significant correlation between the levels of skin AGE and facial wrinkling was also observed. The facial skin AGE data may provide new insight into skin aging research.

INTRODUCTION

Skin autofluorescence (SAF) has been well validated in the past 10 years after the first paper appeared in the literature describing a simple noninvasive method to measure the accumulation of the advanced glycation end-products (AGEs) in the skin (1). AGEs are formed in the tissue via a nonenzymatic glycation process between sugars and proteins and are implicated in the pathophysiology of aging, including complications of multiple aging-related diseases (2–5). With the convenience of the noninvasive measuring technique, skin AGEs have been measured in large study populations. As such, SAF has been established as a noninvasive marker of vascular damage in Caucasians patients with type 2 diabetes (6–10), as a strong predictor of cardiac mortality in diabetes (11), as a complementary test to assess kidney function as well as to predict mortality in hemodialysis patients (12–14), and as a novel risk marker in chronic kidney disease (15). In addition to

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diabetic and renal diseases, elevated levels of skin AGEs have been reported in patients with systemic lupus erythematosus (16), chronic cerebral ischemia (17), schizophrenia (18), peripheral artery disease (19,20), and chronic heart failure (21).

SAF has also been studied in healthy people, and positive correlations of skin AGE levels with chronological age have been established for Dutch and Slovak Caucasians, Japanese, Chinese, and Saudi Arabians (22–26). Because skin pigmentation can influence the results of SAF, corrections for melanin and hemoglobin exist to enable more meaningful correlations with age in those individuals with darker skin pigments (27). In the skin, AGEs are considered photosensitizers and can generate reactive oxidative species on ultraviolet (UV) irradiation, which accelerates the skin aging process (28,29). Meanwhile, there have been reports indicating that chronic UVB exposure induces additional fluorescence excitation bands in mice skin (30) and observations of significantly more AGE staining in sun-exposed skin than in sun-protected skin, suggesting that solar irradiation increases dermal glycation (31). Increased skin AGEs has also been correlated with heavy smokers and chronic obstructive pulmonary disease patients (32).

Of the SAF studies, most of them has used commercially available noninvasive instruments, AGE Reader (DiagnOptics, Groningen, The Netherlands) or SCOUT DS (VeraLight, Albuquerque, NM), to measure the accumulated skin AGEs *in vivo*. Their detailed operating principles were previously reported (1,8). To date, all studies using SAF as a measure of AGEs have been obtained from the volar forearms except two reports in which the measurements were obtained on the inner aspect of the upper arm skin (23,33).

The facial skin has been the primary focus of antiaging research in the skincare industry, and the noninvasive *in vivo* measurement of facial skin AGEs would add new data to aging research. In this study, we report skin AGEs measured from the left-cheek skin and compare the results with that of the left volar forearm skin. Our aim was to show the site and gender differences of skin AGE level and to correlate the results with participants' chronological age as well as the level of facial wrinkling. We selected the left cheek for measurement to maximize the effect of sun exposure on the skin because of the driving convention in the United States.

MATERIALS AND METHODS

SAF MEASUREMENT

A commercially available AGE Reader™ SU (DiagnOptics) was used to noninvasively evaluate the level of accumulated AGEs in the skin. Its measurement principle is based on the properties of SAF because the primary components of AGEs in the skin emit a characteristic SAF when excited by UV light. The instrument illuminates a skin surface area of 4 cm^2, guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation 370 nm). Emission light and reflected excitation light from the skin are measured with a spectrometer in the 420–600 nm range. Because skin pigmentation may absorb light and thus influence autofluorescence, skin reflection measurements across the 300–420 nm range were compared with those of a white Teflon block (1). SAF is calculated as the ratio of the light intensity reflected by the skin in the 420–600 nm wavelength range and the light intensity in the 300–420 nm wavelength range and is represented as the skin autofluorescence ratio (AFR) in AGE Reader (1).

The default design of the instrument is to be used on a bench top for ease of measuring the volar forearm. To measure the facial skin, we obtained the instrument manufacturer's technical approval and tilted the measuring surface by a 65° angle, thus allowing the left cheek of a subject to comfortably engage the measuring window. Figure 1 shows the configuration of the new installation together with a picture of the traditional position.

FACIAL SKIN COLOR AND WRINKLE MEASUREMENTS

Facial skin color and wrinkle measurements were performed by using image analysis means. VISIA-CR® (Canfield Scientific, Parsippany, NJ) was used to capture facial images under five different lighting conditions (standard, flat, UV, cross polarized, and parallel polarized). Amway exclusive Facial Analysis Computer Evaluation System was used to objectively measure skin color parameters such as individual typology angle (ITA°) and facial wrinkles. The wrinkle measurement was from the frontal image of whole face, and the ITA° was measured from the cheek area (37). Figure 2 shows a sample output of wrinkle analysis result (A) and a region of interest on a facial image for skin color measurement (B).

SUBJECTS

A total of 239 healthy Caucasian volunteers, Fitzpatrick Skin Type II and III, aged 21- to 65-year old with 151 females (63.2%, mean age = 43.1) and 88 males (36.8%, mean age = 41.4) participated in the study. All subjects were provided with verbal and written descriptions regarding the intent of the study and each signed an informed consent form consistent with the requirements in the Code of Federal Regulations Title 21 (21 CFR)

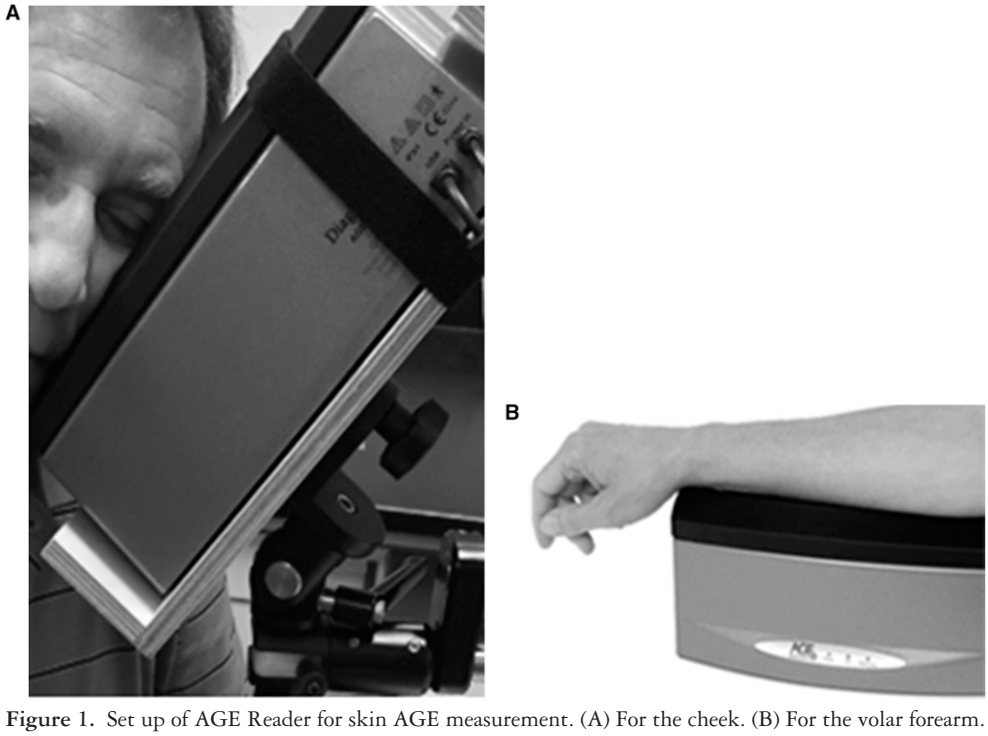

Figure 1. Set up of AGE Reader for skin AGE measurement. (A) For the cheek. (B) For the volar forearm.

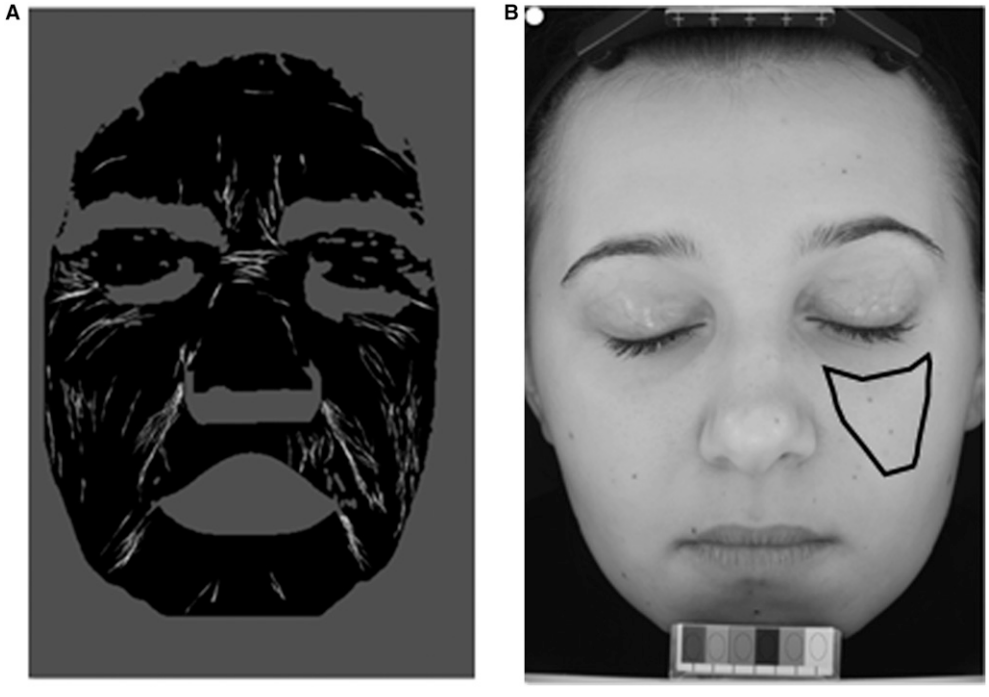

Figure 2. Illustration of Image analysis methods. (A) Output of wrinkle analysis. (B) Region of interest on the left cheek for color analysis.

50.25 [see footnote for institutional review board's (IRB) review of the study]. Subjects were instructed to cleanse their skin and acclimatize for 15 min in a temperature and humidity controlled lab ($T = 21° \pm 0.5°C$ and $RH = 45\% \pm 1$). SAF measurements were taken on the left cheek and on the left volar forearm with triplicate measurements on each site.

STATISTICAL ANALYSIS

The mean value of triplicate measurements was used to construct scatter plots for male and female groups of the cheek and forearm skin sites. Correlations of subjects' skin AGE level, and their chronological age were obtained by using the best-fit model of least-square regression analysis. The statistical significance of the correlations between AGE and chronological age, wrinkles, and ITA° was assessed using the *F*-test at the 95% confidence level. The difference between face and arm, as well as between male and female, was examined using the Student *t*-test. The rate of change in skin AGE levels with age was calculated for each gender and skin site from the best-fit model.

RESULTS AND DISCUSSION

COMPARISON OF SKIN AGE LEVELS BETWEEN FACE AND ARM

Overall, the level of skin AGEs measured from the left cheek was lower than that of the volar forearm ($p < 0.001$) as illustrated in Figure 3 in which trend lines of the best-fit

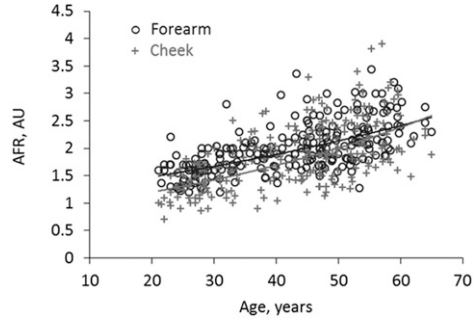

Figure 3. Comparison between cheek and forearm skin AGEs in a Caucasian population of men and women. The AGE level in the facial skin, as represented by the skin AFR, is significantly lower than that of the forearm in young people ($p < 0.001$). It increased with age rapidly, reaching nearly the same values as that measured in the forearm skin of subjects older than 60 year of age. Dark line and circles = the AGE levels in the left volar forearm; gray line and crosses = AGEs in the left cheek.

model for both skin sites are shown. From the trend lines, it is clearly seen that the AGE levels in the cheek skin are much lower than that of the forearm skin in young people, but the difference becomes smaller as the subjects increase in age. Dividing the subjects' age into subgroups by decades, a trend of diminishing statistical significance of the site difference over age is shown in Table I. As we can see from the column for both genders, the facial skin AGE levels were significantly lower than the arm in the age groups up to 35–45 year old.

GENDER DIFFERENCE OF SKIN AGE LEVELS BETWEEN FACE AND ARM SITES

Separating the skin AGE results by gender demonstrates the unique gender difference between face and arm skin sites. As can be seen in Table I, in the "all ages" row, the mean level of AGEs in the facial skin was significantly lower in men than in women with AFR values being 1.648 ± 0.52 and 1.946 ± 0.59, respectively ($p < 0.001$). However, this gender difference did not exist at the forearm site (1.925 ± 0.40 vs. 2.034 ± 0.48, $p > 0.05$). In addition, statistically significant site difference of AGE level is seen in men's age groups of up to 35–45 year of age, whereas it is only up to the 25–35 age group for

Table I
Differences between Cheek and Forearm Skin AGE Levels in Age Brackets of Decades

	Both genders			Men			Women		
	Forearm	Cheek	*p* @95% CL	Forearm	Cheek	*p* @95% CL	Forearm	Cheek	*p* @95% CL
All ages	1.994	1.849	0.000	1.925	1.684	0.001	2.034	1.946	0.159
Age ≤ 25	1.546	1.187	0.002	1.525	1.134	0.012	1.559	1.219	0.020
25 < age ≤ 35	1.688	1.455	0.000	1.668	1.274	0.000	1.700	1.560	0.036
35 < age ≤ 45	2.001	1.815	0.014	1.954	1.742	0.028	2.050	1.891	0.272
45 < age ≤ 55	2.113	2.092	0.707	2.045	1.894	0.206	2.151	2.200	0.586
55 < age ≤ 65	2.474	2.344	0.163	2.484	2.374	0.600	2.471	2.336	0.402

p: probability value; CL: confidence level.

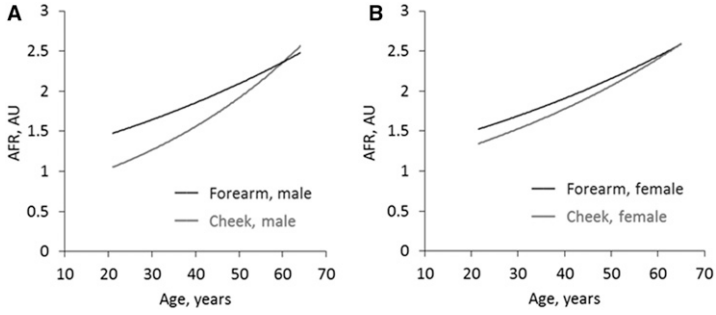

Figure 4. Differences between the trend lines of cheek and forearm skin AGEs as a function of age in the male and female groups. (A) Skin AGE level in the male group. Gray line = cheek; dark line = forearm. (B) Skin AGE level in the female group. Greater difference in skin AGE level is seen between the cheek and forearm sites in men than in women.

women. Plotting the trend lines of skin AGEs by gender illustrates the difference visually. The gap between the trend lines of face and arm is much wider in men (Figure 4A) than in women (Figure 4B). The trend line for the cheek skin AGEs in Figure 4A was steeper than that in Figure 4B because of a significantly lower level in young male groups. In fact, for men in the group of <25 year of age, the facial AGE level was measured to be ~65% of the forearm skin. It increased quickly until attaining a nearly equivalent or greater level as in the forearm skin by the time the men reached 60–65 year of age. Interestingly, this dramatic change in facial skin AGE level is not seen in the female group. In young women, although still statistically significant, the facial SAF measures were only slightly lower than what was measured from the forearm skin (Figure 4B).

To further describe the site and gender differences, we calculated the rate of change from the trend lines in Figure 3. Data shown in Table II indicate that the facial skin AGEs in men increased with age exponentially in a rate of 0.021 (log AFR units per year), in contrast to a 0.015 rate for women—a 1.4 times higher rate for men than that of women. Critically, the rate of change in skin AGEs at the facial site of men was a full 1.75 times the rate of change measured at the forearm site of both men and women (0.012 log AFR units per year).

EFFECT OF FACIAL WRINKLES ON AGE

Facial wrinkles quantified by image analysis were correlated with cheek area AGE levels. Wrinkles were seen to increase with increasing AGE levels on the cheek. This trend was statistically significant ($p < 0.05$) as shown by Figure 5.

Table II
The Rate of Change of Skin AGEs with Age

	Left cheek	Left volar forearm
Male	0.021	0.012
Female	0.015	0.012
All subjects	0.017	0.012

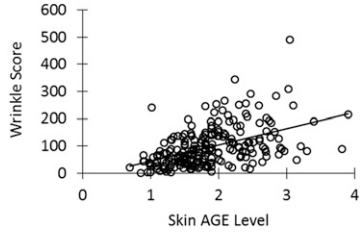

Figure 5. Correlation between skin AGE level and facial wrinkles. Facial wrinkles increased with the increase in skin AGE levels. The correlation was statistically significant.

INFLUENCE OF PIGMENTATION ON SKIN AGE LEVEL

Skin pigmentation potentially affects the result of SAF measurement. In AGE Reader, this effect is automatically corrected so that the AGE measurement is independent of skin pigmentation level. To verify this point, we measured skin color parameters (L^*, a^*, b^*) in CIELAB color space from the cheek area of the facial image and calculated ITA° to correlate with the AGE levels in the same area. The result is shown in Figure 6, where no correlation is exhibited between the two skin parameters.

DISCUSSION

The level of facial skin AGE measured noninvasively using bioinstrumentation is, to the best of our knowledge, the first such data to appear in the literature. It adds new data to SAF study as well as antiaging skincare research. In this study, we observed the association between the increased facial wrinkling and skin AGE level, which suggests the use of anti-glycation technology may be a meaningful antiaging strategy.

It was interesting to see the difference between the AGE levels in face and in inner forearm skin. One would expect the levels of skin AGEs in the left cheek to be higher than that of the volar forearm, because it is an area that gets more sun exposure naturally in one's daily life. The results we obtained are opposite this hypothesis. Consistently, lower levels of AGEs across the majority of the age span were shown on the face than on the forearm. Further studies are needed to understand the phenomenon. The second fact we observed was that the facial AGE level in men was lower than in women; whether it was due to the difference in gender or in lifestyle remains to be elucidated. The observation that we found most interesting was the difference in the rate of change in AGE accumulation in the facial skin between genders and skin sites. The facial skin of men showed

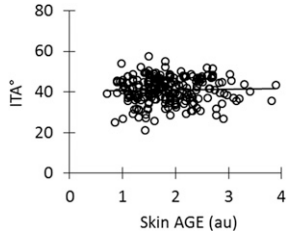

Figure 6. Correlation between skin AGE level and skin pigmentation. The r^2 was 0.0006 ($p = 0.726$), virtually no correlation between these two skin properties.

highest rate of change among all conditions studied. It may owe to the difference in life style—males possibly being more active in outdoor activity with less conscientious daily skin care. Nevertheless, these results of facial skin AGEs open up new research directions in skin aging and anti-glycation studies.

CONCLUSION

This study shows for the first time the *in vivo* skin AGE data of the human facial skin as measured by autofluorescence using a noninvasive technique. It adds new data to skin aging research. The low AGE level observed in the facial skin did not support the reported phenomenon that solar irradiation increases dermal glycation unless a significant difference in antioxidant activity is evident (34-36). The faster increase in AGE level in the facial skin of men may be due to their lack of adequate skin care when compared with women. Further study in the difference of antioxidant activity between genders may help elucidate the mechanisms of facial skin glycation and aging process.

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Footnote: IRB review of the study.

IRB review of the study: This was a skin assessment study conducted on volunteers using a medical device for diagnostic use that has been deemed of no significant risk. As per the Food and Drug Administration Information Sheet Guidance "Significant Risk and Nonsignificant Risk Medical Device Studies," the following exemption for IRB approval applies to this study: …. diagnostic device studies (e.g., in vitro diagnostic studies) are exempt from the requirements of 21 CFR Part 812 under certain circumstances. The study is exempt as long as the sponsor complies with the requirements at 21 CFR 809.10(c) for labeling, and if the testing: (i) is noninvasive; (ii) does not require an invasive sampling procedure that presents significant risk; (iii) does not by design or intention introduce energy into a subject; and (iv) is not used as a diagnostic procedure without confirmation of the diagnosis by another, medically established diagnostic product or procedure. 21 CFR 812.2(c)(3).

Therefore, while we followed guidelines of good clinical practices including informed consent and appropriate legal oversight, we did not seek an IRB approval as per the previously mentioned exemption.