SO WHY DON'T I LOOK YOUNGER?

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ABSTRACT

The Asian population is often considered to age better than Western counterparts. We studied a Western cohort of subjects in California and a Japanese cohort recruited in Tokyo, recording their chronological age as well as their 'apparent age'. The apparent age of all subjects was assessed based on how old they looked as a result of dermatology clinicians grading key facial aging parameters such as pigmentation and age spots. In the U.S. population, we found a spread in the error of age assessment (the numerical difference of apparent age and chronological age) of 16 years, with the average at +/- 7 years. Data from a follow-up study with a Japanese cohort showed a similar pattern, but exhibited a smaller range in the average error in age assessment. In the Japanese population, subjects' appearance, on average, ranged from 6.2 years older than their chronological age to 4.5 years younger than their chronological age. Several biological parameters were measured and a correlation with apparent age was found: those subjects who appeared older for their chronological age had elevated levels of PGF2α isoprostanes, known to be in vivo biomarkers of oxidative stress. Conversely, subjects who appeared younger than their biological age had correspondingly lower levels of these markers. In a third study, oral supplementation targeting the antioxidant defense network using normal and oxidatively-stressed (smoking) subjects showed an improvement in skin aging attributes among oxidatively-stressed subjects receiving supplementation. Overall, these studies demonstrate a link between how old someone appears and the endogenous level of oxidation. Additionally, we found that nutritional supplementation of subjects with high levels of oxidative stress (smoking) resulted in an improvement in skin aging attributes.

INTRODUCTION

Chronological age may not be accurately reflected by the appearance of one's skin. Some people appear older than their chronological age, while others appear younger then their chronological age. Skin aging is affected by the interaction of extrinsic factors like diet, behavior, and environment on the predominant intrinsic factor, the genome. Genetic expression, or the response of the genome to both negative and positive extrinsic factors, yields either a more or less favorable appearance aging trajectory. Oxidative stress to the epidermis, which is predominantly from UV exposure (1,2) but can also be caused by pollution (3) and habits such as smoking, is often studied at the molecular level and has been found to contribute to aging, DNA mutagenesis, inflammation, and other harmful processes. Dietary components supply antioxidants, that can help reduce and attenuate oxidative stress (4), thus potentially having a beneficial effect on the aging appearance trajectory. Various deficiencies in the typical Western diet may be corrected using nutritional supplementation, thus providing additional key antioxidants (5).

METHODOLOGY

STUDY I—In an IRB-approved study based in the United States, 25 female Caucasian subjects between 45 and 65 years of age with no history of cosmetic surgery or current use of cosmetically active ingredients were recruited for participation. Photographs were taken using the VISIA Complexion Analysis System and graded by five independent clinicians to assess subjects' apparent age and skin attributes. Serum samples were obtained and analyzed for NADH oxidase, known to increase superoxide (6).

STUDY II—For this Japan-based IRB-approved study, 150 Japanese women between 45 and 60 years of age were recruited. By personal interview, two groups of subjects were selected—those who looked younger and those who looked older for their chronological age. Three photographs of each subject in these sub-groups were taken and evaluated by three Japanese dermatologists that were blinded to the subjects' chronological age. Skin appearance was graded and apparent age was estimated. Blood plasma and serum were collected for oxidative stress evaluation. Isoprostanes, considered to be the "gold standard" biomarker of oxidative stress in vivo (7), were measured as per Taylor and Traber (8). Briefly, 15R-8-isoPGF2α and PGF2α were quantified by a combination of solid phase extraction and liquid-liquid extraction, followed by HPLC-tandem mass spectrometry. Another marker of oxidative stress was estimated as thiobarbituric acid-reactive (TBARS) malondialdehyde-like materials (9).

STUDY III—A US-based, IRB-approved, single blind, placebo-controlled study of female smokers (n=13) and non-smokers (n=17) from 50 to 70 years of age was conducted. For 12 weeks, subjects took a daily oral dose of vitamins A, C, E, polyphenols, and carotenoids. Additionally, as an unsupplemented control group, healthy non-smokers between 18 and 30 years of age (n=20) were recruited. Facial photographs at baseline and 12 weeks were taken using the VISIA Complexion Analysis System and scored by three blinded dermatologists on clinical parameters of skin aging.

RESULTS AND DISCUSSION

STUDY I

Fifteen women were estimated to be older than their chronological age with a mean error in age assessment (apparent age minus chronological age) of 7.38 years older, based on an assessment of their facial skin appearance. Nine women were estimated to be younger than their chronological age with a mean error in age assessment of -7.94 years (younger). Overall skin health, deep wrinkles, fine wrinkles, skin color tone, skin laxity, fullness of the face, and overall skin health correlated with the apparent age while pore size, evenness of color, darkening below the eyes, and droop of the eyelids did not (Table I). However, only darkening below the eyes correlated with subjects' chronological age. Mean error in age assessment was positively associated with NADH oxidase where those appearing older than their chronological age were found to have higher serum NADH oxidase levels than those appearing younger than their chronological age (Figure 1).

	Apparent Age (AA)	Chronological Age (CA)
Attribute	p Value	p Value
Deep winkles	<0.0001	0.4
Fine wrinkles	0.0003	0.67
Skin color tone	0.002	0.92
Skin laxity	<0.0001	0.21
Pore size	0.82	0.11
Evenness	0.53	0.82
Darkening below eyes	0.06	0.01
Drooping eyelids	0.12	0.62
Fullness of the face	<0.0001	0.47
Overall skin health	<0.0001	0.80

TABLE I. Correlations between attributes of skin health and age, both apparent age and chronological age, for Caucasian females

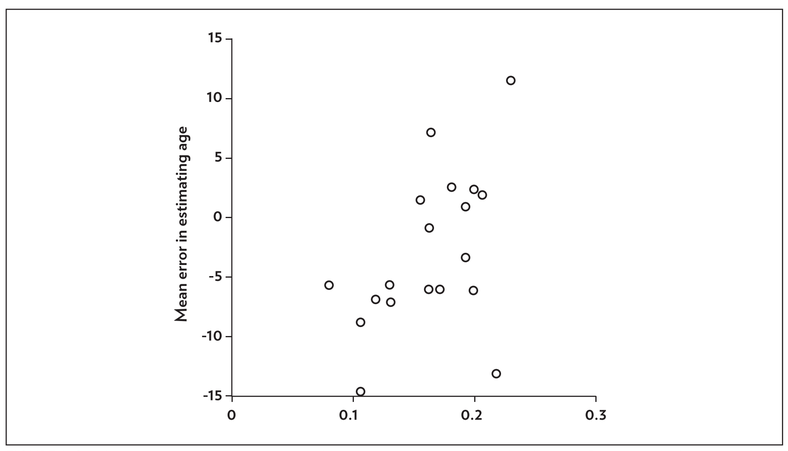

FIGURE 1. Correlation between error in age estimation (apparent age minus chronological age) and NADH oxidase levels (increased superoxide generation) for Caucasian females (relative units)

STUDY II

In the second study, in Japanese women, data indicate that there was a similar discrepancy between chronological age and apparent age based on an assessment of the facial aging features of participants. Additionally, the levels of isoprostanes were elevated in the older-appearing group with PGF2α levels approximately twice those of the younger-appearing subjects (Tables II & III). PGF2α levels positively correlated with an older perceived appearance (Figure 2,7). No correlation was seen between apparent age and UV exposure or consumption of coffee, tea, fruits, vegetables, and meat, or the use of supplements (Table III).

Test Parameter Group	Apparent Age < CA	Apparent Age ≥ CA	Ratio
Error in age assessment (apparent age estimation minus chronological age in years)	- 4.5 ± 1.8 (n=18)	+ 6.2 ± 2.4 (n=24)	
Total apparent age span	10.7 years		
15R-8-iso-PGF2α (pg/ml)	68.3 ± 14.9	69.8 ± 17.6	1.16
8-iso-PGF2α (pg/ml)	47.7 ± 8.9	64.3 ± 8	1.35
Unknown 1 (pg/ml)	300.2 ± 66.6	394.0 ± 202.8	1.38
PGF2α (pg/ml)	65.2 ± 19.4	136.15 ± 64.1	2.09
TBARS (µmoles/200 µl)	0.219 ± 0.063	0.340 ± 0.134	1.55

TABLE II. Comparison of oxidative markers of stress for subjects with an apparent age less or greater than their chronological age

Characteristic	Skin Age < CA	Skin Age > CA	p Value
Skin age	46.5	57.5	<0.001
Chronological age (CA)	50.9	51.04	0.925
Iso_PGF2α	47.7	64.27	<0.001
PGF2α	65.,19	136.18	<0.001
BMI	22.81	20.92	<0.0365
UV exposure	0.05	0.38	0.095
Coffee intake/day	1.0	1.04	0.87
Tea intake/day	1.25	1.23	0.94
Oral supplement use	1.10	0.72	0.39
Fruits and vegetables intake/day	1.80	1.69	0.69
"Yes" for meat intake	80%	88.50%	0.38

TABLE III. Skin aging severity ratings performed by four dermatologists using facial photographs and replicated, with Pearson correlation R ranging from 0.82–0.90

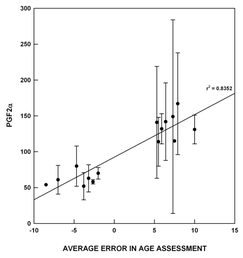

FIGURE 2. Average of error in age assessment [apparent age estimation—chronological age] in years ± standard deviations vs PGF2α concentration, pg/ml.

STUDY III

From the third study, which was designed to evaluate the impact of a targeted nutritional supplement on aging skin appearance assessed by instrumental measurement techniques, only the group of subjects comprised of smokers exhibited an improvement in skin aging parameters (Table IV). UV spots, pores, and elasticity all improved. Dermatologist grading of skin attributes showed an improvement in deep wrinkling and elasticity for the supplemented non-smokers group and improvement in fine lines, deep wrinkling, glow, and hydration in the supplemented smokers group.

RESULTS

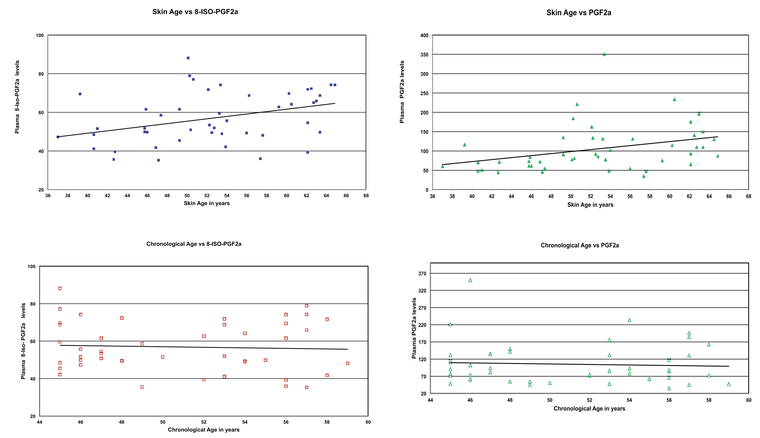

FIGURE 7. Correlation of isoprostane levels with skin age and chronological age (CA)

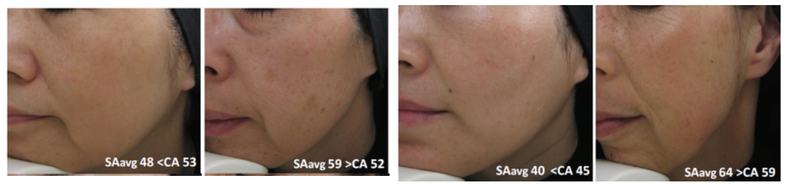

FIGURE 8. Skin age (SA) as compared to chronological age (CA)

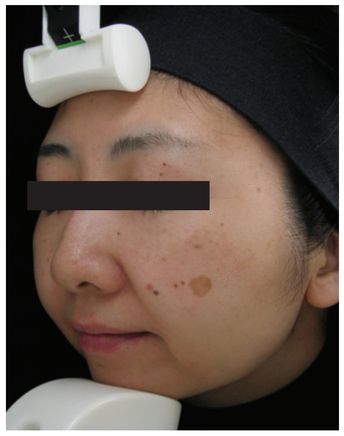

FIGURE 3. S092, chronological age 49, apparent age 39.

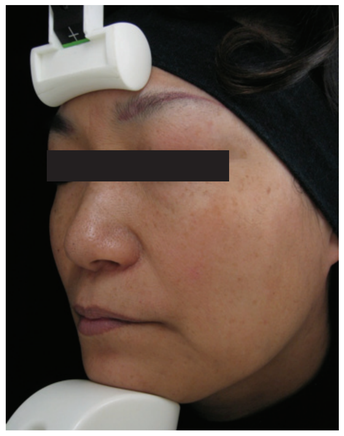

FIGURE 4. S073, chronological age 59, apparent age 50.7.

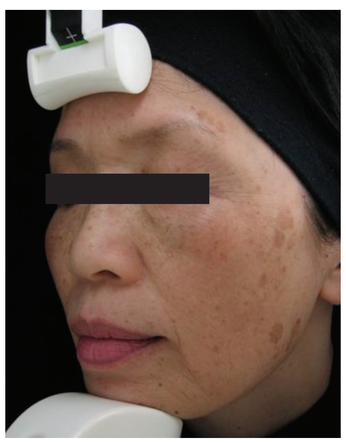

FIGURE 5. S125 chronological age 54, apparent age 62.5.

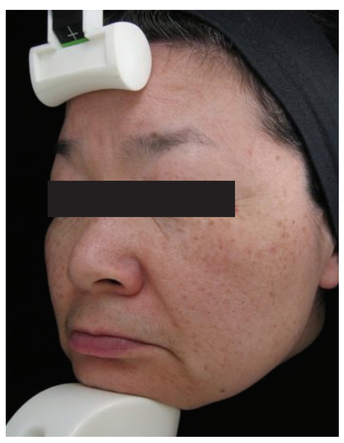

FIGURE 6. S072 chronological age 53, apparent age 64.

Nutritional Supplementation of All Subjects							
Skin Aging Parameter		Nonsmokers N=17			Smokers N=11		
		Mean	SD	P	Mean	SD	P
Assessed by VISIA system	Wrinkling	-4.882	11.050	0.09	+6.615	14.239	0.12
	Visible spots	+1.235	7.471	0.51	+3.154	7.414	0.15
	UV spots	+5.000	28.588	0.48	-17.692	26.196	0.03
	Pores	-0.235	7.496	0.90	+6.385	9.341	0.03
Elasticity (cutometer)		+0.020	0.274	0.77	+0.160	0.270	0.05
Trans-epidermal water loss (g/m2h)		-0.941	5.459	0.487	-0.600	8.515	0.82

TABLE IV. Instrumental analysis of subjects' skin following 12 weeks of nutritional supplementation

Nutritional Supplementation of All Subjects						
Clinical Skin Aging Parameter	Nonsmokers N=17			Smokers N=11		
	Mean	SD	P	Mean	SD	P
Fine wrinkling	+0.235	1.821	0.60	-1.769	2.743	0.04
Deep wrinkling	-1.853	1.579	0.00	+2.500	2.754	0.01
Surface evenness	+0.882	1.996	0.09	+0.154	1.819	0.77
Elasticity	+1.353	1.730	0.01	-1.115	2.468	0.13
Glow	+0.235	3.073	0.76	+2.538	2.570	0.00
Hydration	+0.294	0.985	0.24	-1.846	2.824	0.04

TABLE V. Clinical grading of subjects' skin following 12 weeks of nutritional supplementation

CONCLUSION

There is much interest in how aging affects the facial skin and facial appearance. An 'aged' appearance is often judged by the number and severity of fine lines and wrinkles, sagging, pore size, and changes in skin texture. Using facial skin attributes, we assessed individuals from different populations and lifestyles and estimated their age based on appearance and biochemical parameters. For both cohorts of Caucasians and Japanese subjects, there were groups of individuals who looked much older or younger than their chronological age would predict. A correlation was found between markers of oxidative stress and how old or how young subjects appeared. In our first study, data suggested a relationship between apparent age and oxidative stress in Caucasian subjects. Our second study showed a relation between markers of oxidative stress and the apparent age of Japanese subjects. Our third study demonstrated the ability of a targeted nutritional supplement supporting the oxidative defense network to improve certain skin age-related attributes—most markedly within the high oxidative stress group of smokers. Skin aging, affected by extrinsic factors like diet, behavior, and environment, or intrinsic factors like menopause (10) or genetic predisposition, may benefit from nutritional supplementation targeting a reduction in oxidative stress. Therefore, overall, this work provides further insight into the biology underlying aging skin appearance.

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