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Body mass index, chronological age and hormonal status are better predictors of biological skin age than arm skin autofluorescence in healthy women who have never smoked*

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Summary

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Conflicts of interest

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Background As life expectancy is increasing and healthy ageing becomes more and more important, skin ageing is a growing topic of interest from both a medical and a commercial point of view. The urgency to unravel the causes of skin ageing is rising. However, there is a lack of objective, simple, noninvasive methods to assess biological skin age – a term introduced to describe how old someone looks, covering both the appearance and function of the skin. A rapid, noninvasive assessment of biological skin age would greatly facilitate the execution of the studies required to find the causes of skin ageing.

Objectives To find an objective, easy-to-apply method to assess biological skin age. **Methods** Skin age score (SAS) was compared with skin autofluorescence, a measure of advanced glycation end products in the skin, and several subject characteristics in 32 healthy, white women with little sun-exposed skin and no history of smoking.

Results A moderate, positive correlation ($R^2 = 0.32$, $P = 0.001$) between SAS and skin autofluorescence-based biological skin age was found. However, the variation in biological skin age according to SAS could be explained better by body mass index, chronological age and hormonal status ($R^2 = 0.86$, $P < 0.001$).

Conclusions In the current setting skin autofluorescence did not contribute better to the prediction of biological skin age than chronological age. Biological skin age was best predicted by body mass index, chronological age and hormonal status, and this approach provides a considerable simplification of the application of biological skin age.

What's already known about this topic?

- Objective, noninvasive methods to assess biological skin age are scarce.
- One of the most important characteristics of ageing skin is loss of elasticity, which is associated with dermal advanced glycation end products.
- Skin autofluorescence, measured by the AGE Reader, reflects the amount of advanced glycation end products in the dermal layer of the skin.

What does this study add?

- Skin autofluorescence did not contribute better to the prediction of biological skin age than chronological age in the current set-up with healthy, white women who have never smoked.
- A prediction model including body mass index, chronological age and hormonal status can approximate the biological skin age, and provides a considerable simplification of the application of this biological skin age.

With the increase of the mean age of the population and growing attention to quality of life, skin appearance – and the accompanying ageing of the skin – becomes more and more important. The social relevance of this appearance is emphasized by the growing demand for cosmetic antiageing products, resulting worldwide in a multibillion-dollar skincare business.¹ From a medical point of view, skin ageing has become even more important: it results in dry, vulnerable skin with reduced elasticity and susceptibility to skin disorders like skin cancer, ecchymoses, pruritus and seborrhoeic eczema.² Particularly, the increasing incidence of skin cancer is alarming.³ Knowledge about causes of skin ageing, apart from chronological age, is crucial to be able to treat or even prevent its accompanying skin disorders.

Skin ageing is caused by both intrinsic and extrinsic factors, causing some people to look old and others to look young for their age.^{2,4} To describe the distinction between chronological age and how old someone looks, the term biological skin age was introduced, covering both the appearance and function of the skin.⁵ Genes are thought to determine the rate of intrinsic skin ageing, whereas mainly sun exposure and smoking induce extrinsic skin ageing.^{6–8} Extrinsic skin ageing has been studied widely, whereas the search for specific genes affecting skin ageing has started only recently.^{6–10}

A major issue in studying biological skin age is the lack of objective, noninvasive measurements, specifically of intrinsic skin age. A gold standard is not available. As a result, a large variety of methods have been used to assess skin age in previously conducted studies.^{11,12} In 2002 a validated skin age score (SAS) for women was developed by Guinot *et al.*, in which assessment of 24 characteristics of the skin of the face provides a global skin ageing score.⁵ Up to now, SAS seems to be the most accurate method to determine biological skin age. To unravel the genetic causes of skin ageing, it would be interesting to correlate SAS to genetic profiles. This would be a step forward towards prediction of, and possibly even intervention in, skin ageing. However, the assessment of SAS is quite time consuming and hence a burden for both patient and investigator, specifically in large study populations, as is the case in genetic studies. It would be desirable to have an easier method to assess biological skin age.

The loss of skin elasticity during ageing is related to the formation of advanced glycation end products (AGEs)^{13,14} by the nonenzymatic reaction between free amino groups in proteins and reducing sugars. During the lifetime, AGEs accumulate in the skin, as well as in other organs. AGE formation is increased with oxidative stress and in the presence of diabetes, renal dysfunction or hypertension.^{15–18} The AGE Reader, an instrument developed by DiagnOptics Technologies (Groningen, the Netherlands), provides a rapid, noninvasive quantification of skin autofluorescence (SAF) on the inner forearm, which correlates with the number of several AGEs in the upper dermis.^{19,20} AGE Reader measurements might therefore be capable of determining facial skin ageing in individuals with little sun exposure.

The main goal of this study was to explore whether objective methods such as SAF of the inner forearm, measured with

the AGE Reader, contribute to an accurate determination of biological skin age, which is easier to apply than SAS itself.

Materials and methods

Study design and population

In this cross-sectional study, healthy, female volunteers aged 30–70 years were recruited among hospital staff and visitors at the outpatient departments of plastic surgery and dermatology at the University Medical Center Groningen, the Netherlands. The study was approved by the institutional ethics board and conducted according to the Declaration of Helsinki principles. All subjects provided written, informed consent.

Subjects reporting factors known to influence skin ageing or its evaluation were excluded.² This included skin phototypes V and VI (according to Fitzpatrick's classification), a history of smoking, a body mass index (BMI) < 18.5 or > 25.0 kg m⁻² and a history of facial cosmetic treatment. Subjects with the following concomitant diseases were also excluded, because of their known association with high SAF values: diabetes mellitus, clinically relevant cardiovascular or renal disease, osteoporosis and Alzheimer disease.²¹ Furthermore, subjects with mainly extrinsically aged skin were excluded, based on assessment with SCINEXA.²² To obtain an age-stratified cohort, recruiting continued until four subjects could be included in each age group of 5 years.

Skin assessments

Subjects were clinically evaluated by the first author between 27 June 2013 and 13 January 2014. Subjects were asked not to apply any make-up or skincare products on the evening prior to and the day of the study. At the start of the evaluation, the facial skin was cleaned using a wet tissue, and room temperature and relative humidity were measured.

Next, a questionnaire was completed, with additional information about BMI, skin phototype, comorbidities, hormonal status (Table 1) and the use of skincare products. The extent of extrinsic skin ageing was determined by SCINEXA.²²

Table 1 Subjects' characteristics (n = 32)

Chronological age (years), mean ± SD	49 ± 12
Sex female, n (%)	32 (100)
Body mass index (kg m ⁻²), mean ± SD	22.3 ± 1.2
Skin phototype, n (%)	
I	0
II	16 (50)
III	13 (41)
IV	3 (9)
Hormonal status, n (%)	
0: Premenopausal	16 (50)
1: Pregnant	1 (3)
2: Irregular periods	3 (9)
3: Postmenopausal	12 (38)

Subjects with SCINEXA ≥ 2 , which indicates mainly extrinsically aged skin, were excluded from further investigations and analyses.

Subsequently, SAS was calculated, based on the clinical evaluation of Guinot's 24 characteristics of facial skin appearance.⁵ Finally, SAF was measured three times on the right inner forearm with the AGE Reader. The AGE Reader detects fluorophores that are excited mainly in the range 350–400 nm and have a fluorescence signal between 420 and 600 nm. More details are given by Koetsier *et al.*²⁰

Statistical analyses

Statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NY, U.S.A.) and Minitab 16 (Minitab, Coventry, U.K.). Subjects' characteristics were described using mean $\pm$ SD or proportions and confidence intervals. SAF was based on the mean of three AGE Reader measurements or on the median if the SD/mean was ≥ 0.125 . Ultraviolet reflectance values $> 10\%$ were accepted in order to avoid any influence of skin colour on the results. SAF-based biological skin age was calculated using the best-fit line of SAF values as a function of chronological age. Mutual Pearson's correlation coefficients were computed to assess the relationship between SAF-based biological skin age, SAS, SCINEXA and chronological age. A prediction model for SAS was subsequently determined by univariate and multivariate linear regression analyses using the method of least squares. When appropriate, higher-order polynomial regressions were applied to find significant contributions of higher-order terms of variables. The predicted R^2 was calculated in Minitab, based on the removal of one value at a time and using the other 31 to predict the removed value. $P < 0.05$ was considered statistically significant.

Results

Subjects

Thirty-six subjects were considered for inclusion. Four had to be excluded based on SCINEXA, resulting in 32 included subjects. Their characteristics are shown in Table 1.

Skin assessments

The investigations were performed under controlled temperature (range 21–24 °C) and relative humidity (range 35–65%).

The mean SAS was 48.4 ± 11.8 years. The absolute differences between SAS and chronological age ranged from –13.0 to 8.9 years. There was a highly significant, positive correlation between SAS and chronological age ($R^2 = 0.82$, $P < 0.001$) (Fig. 1). The distribution of values around the mean increased at higher chronological age. In a logarithmic presentation, the deviations around the curve became independent of chronological age. Mean SAS was higher in the subgroup of postmenopausal women than in premenopausal women (61.0 ± 5.0 vs. 40.5 ± 7.0 years).

Fig 1. Linear relationship between skin age score and chronological age. Diamonds represent individual subject scores.

Fig 2. Linear relationship between skin autofluorescence (SAF) and chronological age (continuous line), and reference fit of SAF values (discontinuous line).²² Diamonds represent individual subject scores. AU, arbitrary units.

Mean SCINEXA scores ranged from 1.02 in the youngest age group to 1.58 in the oldest age group, with an overall mean of 1.24 ± 0.37 . SCINEXA correlated poorly with SAS ($R^2 = 0.18$, $P = 0.017$).

The mean SAF was 1.63 ± 0.34 arbitrary units. All ultraviolet reflectance values were $> 10\%$. SAF increased significantly with chronological age. In this study group, the best-fit line of SAF was $0.0183x + 0.731$ ($R^2 = 0.38$, $P < 0.001$), where x is chronological age, as shown in Figure 2. The SD around this fit was 16%, independent of chronological age. Figure 2 also shows the reference fit of the AGE Reader, which was based on values for a mixed group of smokers and nonsmokers.²⁰ SAF-based biological skin age was calculated from the above-mentioned fit formula $[(SAF - 0.731)/0.0183]$, resulting in a mean SAF-based biological skin age of 49.0 ± 18.7 years. The absolute difference between SAF-based biological skin age and chronological age ranged from –31.9 to 36.9 years.

The absolute differences in biological skin age obtained with SAS and predicted by SAF ranged from –36.8 to 38.3 years. The correlation between SAS and SAF-based biological skin age is displayed in Figure 3 ($R^2 = 0.32$,

Fig 3. Linear relationship between skin autofluorescence (SAF)-based biological skin age and skin age score. Diamonds represent individual subject scores.

$P = 0.001$). SCINEXA did not correlate with SAF ($R^2 = 0.09$, $P = 0.099$).

Regression analyses

The effects of possible predictors on biological skin age, measured with SAS, were determined by univariate and multivariate linear regression analyses. Because of the divergent spread of SAS values around the mean at higher chronological age, a logarithmic transformation of SAS and chronological age was applied. Univariate linear regression analyses of $\ln(\text{chronological age})$, SAF, skin type, BMI and hormonal status (Horm) on $\ln(\text{SAS})$ were performed. Graphical presentations of $\ln(\text{SAS})$ as a function of BMI or Horm showed nonlinear relationships. Application of the significant higher-order terms led to much better correlations. The univariate variables $\ln(\text{chronological age})$, BMI, Horm, the second- and third-order terms of BMI and Horm, and SAF, each with sufficient correlation ($P < 0.1$), were included in the multivariate regression analysis. SAF and skin type were not significant predictors of $\ln(\text{SAS})$. No significant interaction effects between the above-mentioned variables were found. The coefficients of the final prediction model are given in Table 2, and biological skin age can consequently be calculated using the following formula:

$$\ln(\text{BiolAge}) = 9.816 + 0.738 \times \ln(\text{Age}) - 0.597 \times \text{BMI} + 0.000398 \times \text{BMI}^3 + 0.00150 \times \text{Horm}^3,$$

where BiolAge is the biological skin age found with the model and Age is the chronological age. The high adjusted R^2 (0.86) and predicted R^2 (0.83) show the strength of this model to predict biological skin age in a comparable population.

Discussion

The present study describes the search for an objective, easy-to-implement and noninvasive method to assess biological skin age. Up to now, SAS has represented the best available instrument for this purpose. We therefore chose to compare

Table 2 Linear prediction model for biological skin age, measured with skin age score (SAS) ($n = 32$)

Predictors	B	95% confidence interval
Constant	9.816	2.148 to 17.483
$\ln(\text{chronological age})$	0.738	0.474 to 1.002
Body mass index	-0.597	-1.111 to -0.083
(Body mass index) ³	0.000398	0.000061 to 0.000735
(Hormonal status) ³	0.00150	-0.000105 to 0.00310

Dependent variable is $\ln(\text{SAS})$.

SAS with biological skin age based on the autofluorescence measurement of the AGE Reader: SAF-based biological skin age.⁵ The main finding of this study was that the AGE Reader result, obtained on skin little exposed to the sun, could not contribute to an accurate determination of biological skin age in a cohort of healthy, white women who had never smoked. The most accurate prediction of biological skin age with the available variables in this study was made by BMI, chronological age and hormonal status.

In regression analysis, chronological age turned out to be the main predictor of SAS. This was reflected in the much higher correlation between SAS and chronological age, compared with the correlation between SAS and SAF-based biological skin age, indicating that chronological age itself provides an even better explanation for the SAS than SAF-based biological skin age. A comparable high correlation between SAS and chronological age was found by Guinot *et al.*⁵ in the development process of SAS and is therefore not a surprising finding, as SAS was developed using only variables that had a linear link with age. Furthermore, while some people look older than their chronological age and some people look younger, one can expect the average to look similar to their chronological age.

A high correlation between biological skin age and chronological age has previously been found using other methods to measure biological skin age, such as perceived age or photographic reference scales.^{23,24} However, Guinot *et al.* showed that the correlation was linear only within the age range of 30–70 years, and it bends off beyond this range. This was explained by the fact that below 30 years of age, age-related changes in the skin were subclinical and not yet reflected in appearance, whereas above 70 years of age the 24 clinical characteristic of SAS were present in almost all subjects, resulting in the same maximum calculated biological skin age.⁵ Our study population was therefore limited to the age range 30–70 years.

Besides chronological age, BMI and hormonal status explained part of the variability in SAS. All three variables have a positive effect on SAS, which means that SAS is higher when these properties increase. Regarding chronological age, this is obvious. However, for the effect of BMI, conflicting results were found in the literature. Guinot *et al.*⁵ found that the effect of BMI was dependent on hormonal status. Rexbye *et al.*⁷ stated that a lower BMI results in a higher perceived

age. Guyuron *et al.*⁸ found chronological age to influence the effect of BMI: a higher BMI gives an older appearance in women aged < 45 years, while women aged > 45 years are said to look younger when their BMI increases. Interactions of BMI with chronological age and hormonal status were not found in our study, indicating that the effect of BMI in our study population is independent of chronological age and hormonal status.

Various explanations have been suggested regarding the role of BMI on biological skin age, for example smoothing of the wrinkles by an excess of subcutaneous fat or a direct effect of hormones stored in these fat cells, but none of these stands firm.⁵ It is clear that further research is necessary to elucidate this matter. In our study population postmenopausal women had a higher SAS than premenopausal women, which was even independent of chronological age. Similar results have been presented by other authors, by showing that hormone replacement therapy results in a younger appearance.^{8,25} It is thought that the lack of oestrogens induced by the menopause causes a reduction in dermal collagen and subsequent atrophy of the skin, representing a plausible explanation for the higher SAS in postmenopausal women.^{2,26} The reduction in dermal collagen would, on the other hand, suggest some decrease in SAF in these postmenopausal women, which is in agreement with the findings of Lutgers *et al.*²⁷ SAF values in the present study were higher in this subgroup than in premenopausal women, although the hormonal status did not have an additional effect on SAF after correction for chronological age (data not shown). However, literature concerning SAF and hormonal status is still restricted.

A possible explanation for the inability of the AGE Reader to predict biological skin age in this study population could be the following. Healthy subjects were selected to ensure that AGE accumulation as a result of clinical diseases was minimized, although the absence of subclinical diseases or other internal processes could not be excluded. For instance, in a group of patients with type 2 diabetes mellitus, Gerrits *et al.*²⁸ showed that AGE Reader values could predict complications that developed in a period of 3 years after the measurement of SAF. Hence, the AGE Reader not only describes some of the changes in skin ageing, but it also reflects the results of other, internal, processes.

The healthy study population was in part determined by SCINEXA. To our knowledge, SCINEXA is the only available validated instrument that discriminates between intrinsic and extrinsic skin ageing symptoms and results in a score that indicates the degree of extrinsic skin ageing.²² Several authors have used SCINEXA – or a part of it – as an outcome parameter, but to our knowledge none has used SCINEXA as an exclusion criterion.^{9,29–33} SAF reflected the health of the study population with respect to the accumulation of advanced glycation end products.¹⁹ The SAF values obtained with the AGE Reader in this study population were relatively low compared with reference values determined by Koetsier *et al.*²⁰ in a mixed group of smokers and nonsmokers. In 428 healthy subjects they found a reference fit line of $0.024x + 0.83$, where x

is chronological age in years, compared with $0.0183x + 0.731$ in the present study population. Our fit line was lower even than that found by Koetsier *et al.* in the nonsmokers ($0.023x + 0.83$), which could be explained by the fact that their nonsmokers were only current nonsmokers, and included subjects with a history of smoking, whereas our subjects had never smoked.

Our healthy, homogeneous study population forms a unique aspect of this study. Both the AGE Reader and the SAS have never been used in such a population before. Using this approach enabled us to compare the measurements of the AGE Reader with a purely intrinsic biological skin age, in anticipation of the possible use of the AGE Reader in genetic studies. This unique population could, on the other hand, also have been the cause of the limited correlation that was found between the two biological skin ages measured by SAS and the AGE Reader. This is a limitation of the present study, as the possibility of extrapolation to other populations, for example men, smokers or nonwhite populations, has not been investigated. It is possible that the AGE Reader would better predict biological skin age in populations with more pronounced differences in skin ageing.

In conclusion, biological skin age appeared to be dependent mainly on BMI, chronological age and hormonal status. The AGE Reader, at least in the current set-up, did not contribute to the prediction of biological skin age in this cohort of healthy, white women who have never smoked. Regarding the current population, this study shows that the time-consuming procedure of SAS can be approached using BMI, chronological age and hormonal status, resulting in a considerable simplification of the applicability of biological skin age.

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