

# Dermal Factors Influencing Measurement of Skin Autofluorescence

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## Abstract

**Background:** Skin autofluorescence (SAF) is a noninvasive marker of accumulation of advanced glycation end products. It predicts cardiovascular complications and mortality in diabetes and renal failure. We assessed the influence of potential common confounders in SAF measurement, by determining the effects of endogenous and exogenous local dermal changes by body creams, hyperemia, vasoconstriction, and hydration.

**Methods:** SAF was measured before and after local administration of body lotion, day cream, sunscreen, or self-browning cream and after attempts to remove these effects with alcohol swabs and washing. SAF was measured before and during three hyperemia maneuvers: vasoconstriction and on a dry and wet skin.

**Results:** The body lotion increased SAF by 18%. Day cream, sunscreen, and self-browning cream gave an increase of >100%. Except for body lotion, subsequent cleaning with alcohol swabs and washing with soap did not return SAF to baseline values. The effect of self-browning cream persisted for 2 weeks and that of sunscreen for 4 days. Hyperemia caused by a hot bath, capsicum cream, or postocclusive reactive hyperemia gave a decrease in SAF of, respectively, 18%, 22%, and 2.3%. Vasoconstriction caused by immersing the arm in cold water gave a 10% increase. Hydration state did not influence SAF.

**Conclusions:** Measurement of SAF is strongly affected by several skin creams. This effect was often not fully corrected by alcohol swabs and washing with soap and may persist for many days. Marked hyperemia and vasoconstriction also influence SAF. We advise avoiding these potential error sources.

## Introduction

ADVANCED GLYCATION END PRODUCTS (AGEs) accumulate in tissue over a lifetime, which is regarded as a process of normal aging.<sup>1</sup> It is well known that AGE accumulation is accelerated in diabetes mellitus and renal failure and contributes to long-term complications and mortality.<sup>2–6</sup> AGEs play a major pathogenetic role in many age-related diseases as a result of cross-link formation and activation of cell membrane receptors, including the receptor for AGE, which leads to activation of several oxidative and inflammatory pathways.<sup>7–9</sup>

Accumulation of AGEs in tissue can be assessed non-invasively with a technique named skin autofluorescence (SAF), which uses ultraviolet (UV) light for the excitation of fluorophores in the skin. This technique has previously been validated by simultaneous measurements of SAF and assessments of specific AGEs from skin biopsies of the dermal layer at the same measuring site.<sup>10</sup> Earlier we reported increased SAF in several groups of patients with increased

AGEs formation such as in those with diabetes mellitus, decreased clearance of AGEs such as in those with renal failure, and overt atherosclerotic disease such as in patients with stable coronary artery disease.<sup>5,11,12</sup> Moreover, SAF proved to be a powerful predictor of complications and mortality in diabetes and renal failure.<sup>2,3,5</sup>

Clinical application of SAF to estimate risk in diabetes mellitus and renal failure is gaining ground. For adequate application of SAF it is important to know what factors might influence or disturb the measurement. The effects of excitation wavelength and skin color in general have been previously studied and reported.<sup>13,14</sup> Also, the influence of an AGE-rich meal resulting in a temporary postprandial rise of 10% has been reported by Stirban et al.<sup>15</sup> The present study addresses the influence on SAF measurements of potential common confounders inducing local exogenous and endogenous dermal changes. First, the influences of several skin sun protecting and tanning creams on SAF are evaluated. Second, we measured the effects of hyperemia and vasoconstriction. Third, the effect of wetness of the skin was addressed.

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## Subjects and Methods

### Subjects

Healthy volunteers from the normal population were recruited to participate. Informed consent was obtained. Inclusion criteria were Caucasian race and an age between 18 and 65 years. Exclusion criteria were the use of medication, a prior medical history, or any current medical problem.

### Assessment of SAF

To assess tissue AGE accumulation, SAF was measured with the AGE Reader™ (DiagnOptics Technologies BV, Groningen, The Netherlands). The AGE Reader is a desktop device that uses the characteristic fluorescent properties of some AGEs to estimate the level of AGE accumulation in the skin. Technical details of this noninvasive device concerning the optical technique have been described more extensively elsewhere.<sup>10,14</sup> In brief, the AGE Reader illuminates a skin surface of 4 cm<sup>2</sup> guarded against surrounding light with an excitation light source with a peak excitation of 370 nm. This wavelength is in the UVA spectrum. Emission light in the wavelength range of 420–600 nm (fluorescence), and excitation light that is reflected by the skin with a wavelength range of 300–420 nm from the skin is measured with a spectrometer. SAF was determined from the ratio between the emission light and the reflected excitation light, using the AGE Reader software version 2.2. In the current series of experiments, the forearm was positioned on top of the device in the usual manner as described by the manufacturer. For each SAF value, a series of three consecutive measurements was carried out, which took less than a minute. The mean of these three consecutive measurements was calculated and used in the analyses.

### Study protocol

First, several representatives of dermal creams were tested. Body lotion (Etos®, Beverwijk, The Netherlands), facial day cream (Etos), water-resistant sunscreen with a UVA/UVB protective value of 50 SPF (Etos), and self-browning cream containing dihydroxyacetone (DHA) manufactured by Vichy® (Asnieres, France) were evaluated. Specifications of the contents of the creams are provided in the Supplementary Appendix (Supplementary Data are available at [www.liebertonline.com/dia](http://www.liebertonline.com/dia)). Beforehand, we tested the auto-fluorescent and light-absorbing properties of the creams itself on a calibration material that has zero autofluorescence and a practically 90–100% reflectance. Then, at baseline, a standard triple measurement of SAF was performed before application of the cream on the lower arm of the study subjects. SAF was again measured 5–10 min after application of the cream, permitting time for the cream to fully draw into the skin. After this, SAF was measured after rubbing with an alcohol swipe and subsequently after washing the skin with water and soap in an attempt to clear the skin of the applied cream. Subsequent alcohol swipes or washings were not done in the case of browning cream. Finally, we assessed the natural time for the effect of sunscreen and DHA cream to wear off. We chose these two creams as they proved in previous experience and in the initial steps to have the most potent and persistent effects on SAF. The cream was applied, and subsequently SAF was measured daily with subjects performing their normal daily routine.

Second, we investigated the effect of hyperemia and vasoconstriction. Hyperemia was elicited in several ways. First, the arm was immersed in warm water at 42°C for 4 min. SAF was measured directly after drying the skin with a towel. Second, a tourniquet was applied for 4 min, and SAF was measured during the postocclusive reactive hyperemia/reperfusion phase 2 min after the tourniquet was released. Third, capsicum cream was applied locally on the measurement site for 4 min, which results in a prominent local vasodilatation of the skin within several minutes of application. SAF was measured after removal of the cream. Intrinsic autofluorescence and absorbance effects of capsicum cream are shown below. Vasoconstriction was induced by immersing the arm in cold water of 12°C for 4 min. After the arm was dried with a towel, skin AF was immediately measured.

Finally, the influence of local dermal wetness was addressed by measuring SAF before and directly after application of a wet cotton gauze for 5 min. After removal of the gauze the skin was not dried before SAF measurement.

### Statistical analysis

Data were gathered in a database (SPSS version 15.0, SPSS Inc., Chicago, IL). Normal distribution of the variables was tested by Kolmogorov–Smirnov tests. Descriptive statistics are, therefore, presented as mean with SD in the case of normal distribution and otherwise as median with interquartile range or as number of patients. A paired Student's *t* test was used for normally distributed parameters, and a paired Mann–Whitney U test was used for parameters with a skewed distribution. We performed a power analysis and determined that 11 subjects were needed to detect a 5% difference in SAF ( $\beta = 0.8$ ,  $\alpha = 0.05$ ).

## Results

### Subject characteristics

We enrolled 39 study subjects. All subjects were Caucasian and healthy and used no medication as defined by the inclusion and exclusion criteria. The median age was 31 years. Twenty subjects (51%) were male. Eight subjects (20%) were current smokers. Mean body mass index was 23.4 kg/m<sup>2</sup>. Mean blood pressure was 124/76 mm Hg. At baseline median SAF was  $1.69 \pm 0.33$ , which is in agreement with the expected SAF of 1.69 for this mean age,<sup>16</sup> and mean skin UV reflectance was  $17 \pm 4.9\%$ .

### Effect of different body creams

The intrinsic fluorescent and reflectance properties of the different creams as tested in vitro against the white standard are presented in Table 1. There were major differences in both (auto)fluorescence and reflectance between the creams. Body lotion appeared to have negligible fluorescent properties, but day cream and sunscreen were highly fluorescent. Day cream and sunscreen had very high absorbent properties, resulting in very low reflectance levels of 1.5% and 2.0%, respectively. Self-browning cream and capsicum cream showed moderate autofluorescence and little UV absorption.

The effects of different body creams on lower arm SAF measurements are shown in Table 2. Body lotion gave an 18% increase in SAF. Facial day cream and sunscreen, however, gave a 139% and 111% increase of SAF, respectively. Self-browning body cream containing DHA even gave a tripling in

TABLE 1. MEASUREMENT OF INTRINSIC FLUORESCENCE AND REFLECTANCE OF CREAMS APPLIED ON CALIBRATION MATERIAL WITH ZERO AUTOFLUORESCENCE AND 90–100% REFLECTANCE

	n	Autofluorescence (AU)	Reflectance (%)
Body lotion	2	0.076 (0.034)	79.5 (0.71)
Day cream	4	10.85 (1.32)	1.53 (0.096)
Sunscreen	3	3.13 (1.00)	2.03 (0.40)
Self-browning cream	4	0.90 (0.091)	59.5 (1.00)
Capsicum cream	3	1.03 (0.23)	56.7 (3.2)

Data are given as mean ( $\pm$ SD) values.

SAF. This effect was accompanied by a marked drop in reflectance for facial day cream, sunscreen, and self-browning cream; for these three creams reflectance was lowered from 17% to approximately 3%.

The effects of cleaning the skin by rubbing with an alcohol swab and additional rinsing with water and soap are presented in Figure 1. It proved to be relatively easy to eliminate the effects of body lotion on SAF: the effect on SAF was largely reversed by an alcohol swab. After an additional washing, no difference with the baseline SAF level was found. Alcohol swabs only marginally reversed the increase in SAF induced by the day cream and sunscreen. Even additional careful washing of the arm with water and soap did not result in return of SAF to baseline values. With day cream, alcohol swabs gave a 33% fall of SAF towards baseline levels, and by washing another 17% occurred. After the alcohol swab and washing SAF was still elevated compared to baseline:  $2.47 \pm 0.64$  versus  $1.86 \pm 0.41$  ( $P = 0.002$ ). Sunscreen proved to be the cream with the most persistent effects on SAF. Here, alcohol swabs gave only a 13% fall of SAF towards baseline levels, and additional washing gave another 19%. After the alcohol swab and washing SAF was still elevated from baseline:  $2.42 \pm 0.32$  versus  $1.61 \pm 0.21$  ( $P < 0.000$ ). Reflectance also remained significantly lowered in spite of alcohol swaps and washing for both day cream and sunscreen. We assessed the natural wear-off effect of sunscreen and self-browning cream in three subjects to provide an indication. It took approximately 4 days with sunscreen and 2 weeks with the self-browning cream before SAF had returned to baseline levels (Fig. 2).

#### Effect of vasodilatation and vasoconstriction

The effect of vasodilation on SAF was tested by a hot bath, capsicum cream, and reperfusion after release of a tourniquet.

Results are shown in Table 3. Capsicum cream and a hot bath resulted in a pronounced visual hyperemia, whereas reperfusion did not. SAF was lowered by 22% for capsicum cream and 18% after a hot bath. Reperfusion only resulted in a nonsignificant drop of 2.3% in SAF. During all hyperemia maneuvers, UV reflectance was not affected. Vasoconstriction caused by a cold bath caused a 10% higher SAF and a decrease in reflectance from 16.8% to 14.6% ( $P = 0.009$ ).

#### Effect of hydration state of the skin

We measured SAF on a normal dry skin and subsequently on a wet skin after 5 min of application of a wet cotton gauze in 16 subjects. There was no difference in SAF between a dry and wet skin. On the dry skin SAF was  $1.99 (\pm 0.44)$ , and on the wet skin  $2.02 (\pm 0.50)$  ( $P = 0.57$ ). Reflectance also was not different: 17.9% versus 18.3% ( $P = 0.51$ ).

#### Discussion

Our study aims to clarify pitfalls in the measurement of SAF. We show that both the application of several creams as well as extreme vasodilatation and vasoconstriction at the measurement site may have a major effect on SAF. Creams and extreme vasoconstriction can result in falsely high SAF, whereas vasodilatation causes a lower value. This has to be taken into consideration when an SAF measurement is performed.

#### Creams

The increase in SAF after application of creams can be attributed to several different mechanisms. First of all, a cream may have fluorescent properties of its own as shown in Table 1. Second, some creams like day cream and sunscreen are designed to absorb UVA and UVB radiation to protect the skin against the long-term harmful effects on skin photoaging and the development of skin cancer. SAF is the ratio between the emission light (autofluorescence) and the reflected excitation light (with a peak wavelength at 370 nm in the UVA spectrum). Creams like sunscreen and day cream lead to a severe drop in the reflected excitation light, as shown by the lowering of reflectance. Because autofluorescence occurs by definition at higher wavelengths and, thus, is less influenced, the SAF as a ratio between the two would rise. Similarly, creams that show absorption of the excitation light will cause a lower SAF. Third, creams may cause changes in the skin itself. As for self-browning cream, DHA binds to the stratum corneum, leading to chemical changes. This leads to a brown discoloration of the skin and an increased absorption of UVA

TABLE 2. EFFECT OF THE DIFFERENT CREAMS ON SKIN AUTOFLUORESCENCE

	n	SAF (AU)			Reflectance (%)		
		Baseline	Cream	% increase	Baseline	Cream	% decrease
Body lotion	14	1.81 (0.25)	2.14 (0.40)	18*	16 (5)	14 (5)	12.5*
Day cream	8	1.86 (0.41)	4.45 (0.93)	139*	17 (5)	3 (1)	82.4*
Sunscreen	10	1.61 (0.21)	3.40 (1.16)	111*	17 (6)	2 (1)	88.2*
DHA cream	3	1.95 (0.45)	7.77 (1.63)	298*	16 (6)	8 (3)	50*

Data are given as mean ( $\pm$ SD) values.

\*Statistical significance with a  $P$  value of  $<0.001$ .

AU, arbitrary units; DHA, dihydroxyacetone; SAF, skin autofluorescence.

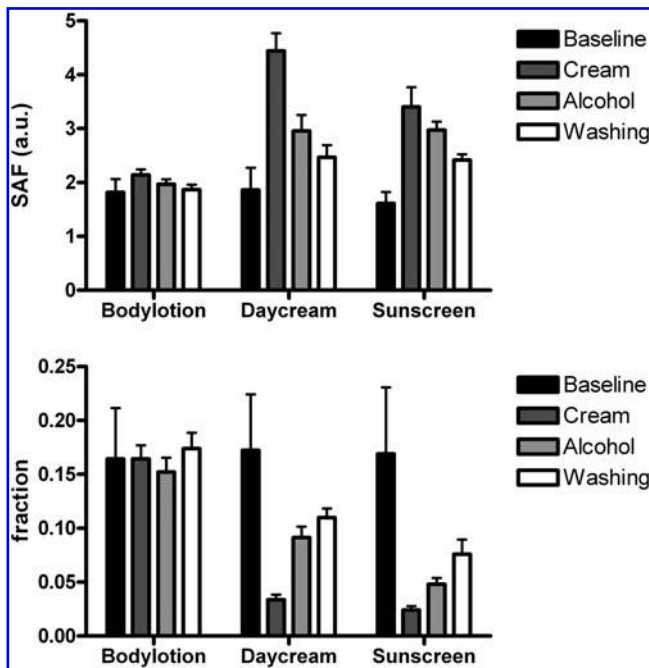


FIG. 1. Effects of cream, alcohol swab, and washing on skin autofluorescence (SAF) and reflectance. a.u., arbitrary units.

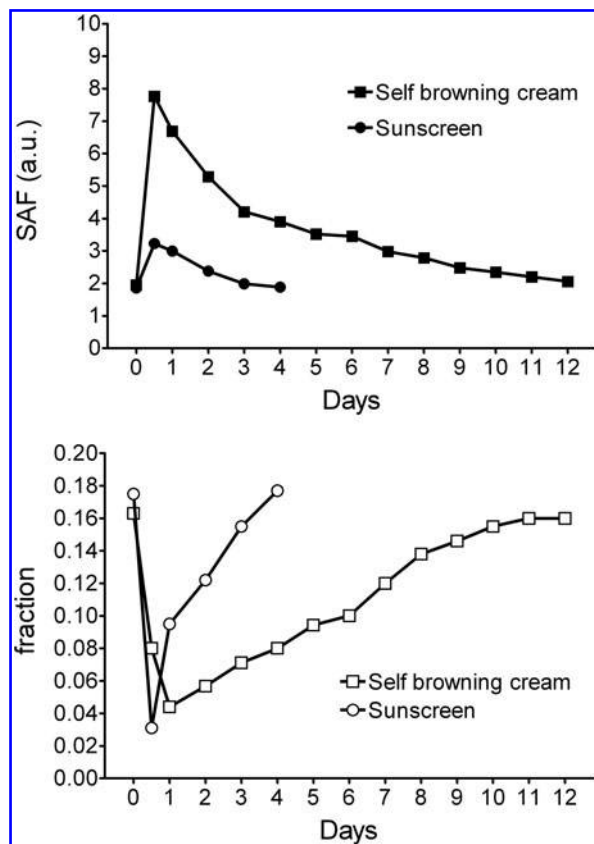


FIG. 2. Wear-off effect of self-browning cream and sunscreen on skin autofluorescence (SAF) and reflectance. a.u., arbitrary units.

TABLE 3. EFFECT OF HYPEREMIA AND VASOCONSTRICTION ON SKIN AUTOFLUORESCENCE

	n	Baseline	Hyperemia/ vasoconstriction	% difference	P
Cold bath	10	1.73 (0.33)	1.90 (0.38)	+10	0.026*
Hot bath	10	1.73 (0.33)	1.41 (0.49)	-18	0.002*
Capsicum cream	12	1.76 (0.38)	1.37 (0.27)	-22	0.002*
Reperfusion	18	1.71 (0.36)	1.67 (0.33)	-2.3	0.11

Data are given as mean ( $\pm$ SD) values.

\*Denotes statistical significance from baseline.

light. As the self-browning cream itself did not show marked intrinsic autofluorescent or absorbent properties, the large effects on SAF are probably explained by these chemical changes in the stratum corneum with resulting fluorescent and absorbent properties. Creams influencing capillary blood flow and hydration state may also result in dermal changes that affect SAF. In conclusion, creams may directly or indirectly influence SAF measurements.

We also showed that the effect of creams on SAF is not completely reversed by alcohol swabs or washing with water and soap. Besides that, it takes 4 days for sunscreen effects on SAF to naturally wear off, and even 2 weeks for self-browning cream. The water-resistant sunscreen we used was designed to not be easily washed off, but it is surprising to see that the effect persisted for 4 days. Self-browning cream containing DHA chemically binds to the stratum corneum. The long delay of SAF to return to baseline levels is therefore mainly determined by the turnover or rather wear-off time of the stratum.

#### Blood flow

Local skin blood flow also influences SAF measurement. This phenomenon can be understood by the fact that hemoglobin has absorbent properties over a broad range in both the excitation and emission parts of the autofluorescent spectrum used during SAF measurements. As an increase in the amount of dermal blood causes lower values of SAF, we may conclude that more of this emission light (fluorescence) is absorbed compared to the absorbance of reflected excitation light. This can also be observed by the virtually unchanged reflected excitation light. Vasoconstriction leads to the reverse effect, in other words, a higher SAF value. In our study, the effects were seen only when extreme vasodilatation was induced. Capsicum cream and the warm bath both led to a clearly visible hyperemia and gave, respectively, 22% and 18% decrease in SAF. Reperfusion after applying a tourniquet only led to a 2% decrease, which was not significant. In this situation, there also was no visible hyperemia. One must keep in mind that the AGE Reader only penetrates the skin to a depth of 0.1–0.2 mm, and therefore only the effect of the most superficial capillaries are measured, mainly nutrient skin capillaries of the upper and lower dermal plexus.<sup>17</sup> This may explain the relatively modest effect of skin blood flow on SAF. With the postocclusive reactive hyperemia procedure, the resulting ischemia may have influenced the NAD/NADH balance. NADH has autofluorescent properties in the same wavelength as AGEs.<sup>18</sup> Surprisingly, during and after ischemia SAF was barely influenced. This may be due to the fact that

NADH fluorescence is found more in the epidermis, where oxygen is provided by direct diffusion from the air, and therefore less influenced by arterial occlusion caused by a tourniquet. We therefore are inclined to believe that NADH barely influences SAF measurement. Vasoconstriction caused by a cold bath of 12°C led to a 10% increase in SAF. We conclude that only extreme vasodilatation and vasoconstriction significantly affect SAF.

### Limitations

We are aware that we only examined a limited number of creams. A list of creams without effects on SAF cannot be provided. Results in the tested creams are, however, very marked and lead to the conclusion that caution in SAF measurements after the local use of any cream is necessary, especially for sun-protecting creams or skin tanners. A low reflectance level in a normal white Caucasian skin should raise the suspicion of the recent use of such a cream. The manual provided by the manufacturer of the AGE Reader, therefore, advises not measuring SAF after recent use of skin creams. Perhaps some other spectral characteristics other than the reflectance level may also become helpful in detection of previous use of cosmetic or other preparations affecting an SAF measurement.

Reperfusion of the arm after application of a tourniquet did not lead to a significant decrease, whereas a warm bath and capsicum cream did. How can this discrepancy be explained? Maybe reperfusion did not lead to enough vasodilatation to cause an effect. Also, measurements were taken 2 min after release of the tourniquet, and capillary flow may have already been past the point of peak reperfusion capillary flow. This may be supported by the fact that a marked hyperemia was seen after both a hot bath and capsicum cream, while this was not visible after the tourniquet release.

Another limitation of our study is that the situations in which we tested hyperemia and vasoconstriction were extreme and do not represent normal physiological situations. Probably the effect of capillary blood flow in normal everyday life will be much less pronounced and may be even neglectable. In previous studies presumed seasonal variations in superficial skin flow were associated with a variation coefficient of 5–6% in SAF.<sup>11</sup>

### Implications

We conclude that a warning not to use any cream in the days before measurement and for sunscreens and sun tanners even in the 2 weeks before measurement seems necessary to assure accuracy. Furthermore, we advise performing measurements with the arm in a normal perfusion state. The manual provided by the manufacturer of the AGE Reader already advises not measuring SAF after recent use of skin creams. Currently, attempts are made to have the device provide warnings about detection of previous possible use of skin creams, using the effects on reflectance and other spectral changes.

In earlier studies the value of SAF in prediction of diabetes complications and mortality has been well established. Also, in dialysis patients SAF has shown predictive value on mortality. When confounding factors like body creams and extreme perfusion states of the measured arm can be cautiously eliminated, the predictive value of SAF may even surpass the earlier reported values.

### Conclusions

This study aims to clarify pitfalls in the measurement of SAF. Local use of body creams can result in falsely high values of the measured SAF, accompanied by a drop in reflectance. In particular, creams that absorb UVA light, chemically react with the stratum corneum, or have autofluorescent properties of their own may affect SAF, persisting for days. An extremely low reflectance in a normal white skin in combination with a high SAF value should raise the suspicion of the recent use of a disturbing cream. Extreme local vasodilatation and vasoconstriction also affect SAF, but to a lesser extent, and should be avoided at the time of measurement.

### Author Disclosure Statement

A.J.S. and R.G. are founders and stockholders of Diag-nOptics B.V., The Netherlands, the manufacturer of the AGE Reader, which has been used as the device for performing skin autofluorescence measurements discussed in this study. M.J.N. and J.D.L. declare no conflicts of interest exist.

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