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# Skin autofluorescence in children with and without obesity

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**Abstract**

**Background:** Obesity is associated with oxidative stress, which is related to increased advanced glycation end product (AGE) formation. AGEs accumulated in skin collagen can be measured with skin autofluorescence (sAF). There are conflicting reports on the influence of obesity on sAF in adults and no data in children. Therefore, this study evaluated sAF in pediatric patients with and without obesity.

**Methods:** In this cross-sectional study, participants aged 4–18 years were included: patients with obesity (body mass index standard deviation score [BMI-SDS] >2.3) and lean controls (BMI-SDS >–1.1 to <1.1). sAF was measured using the AGE Reader®. Participants were stratified according to age (<10, ≥10 to <13, ≥13 to <15, ≥15 to <17 and ≥17 years) and skin type (I–VI).

**Results:** In total, 143 patients and 428 controls were included. In patients, there was no influence of age on sAF ($p = 0.09$). In controls, sAF was higher in children aged <10 years compared to ≥10 to <13 and ≥13 to <15 years ($p = 0.02$; $p = 0.04$). Stratified by age, sAF was higher in patients compared to controls in all age categories, except <10 years of age ($p < 0.01$), while this was not observed when stratified by skin type ($p > 0.05$). Skin type and BMI were significant covariates for sAF.

**Conclusions:** BMI was a covariate for sAF; however, no difference in sAF was observed between children with and without obesity, stratified by skin type. Duration of obesity as well as accuracy of the AGE Reader® might explain this difference. Further research is warranted, in which patients should be matched for age and skin type.

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**Keywords:** advanced glycation end products; children; obesity; skin autofluorescence.

## Introduction

Obesity has become a major health problem, with an ongoing increase in prevalence in both adults and children worldwide [1]. Consequently, obesity-related cardiometabolic complications are nowadays more prevalent and also seen at younger ages [2]. There is however no tool yet to predict the development of obesity-related complications, though advanced glycation end products (AGEs) have been proposed as possible indicators [3, 4].

AGEs are formed endogenously in a non-enzymatic glycation reaction, in which glucose binds irreversibly to proteins or lipids according to the Maillard reaction [5]. In addition, AGEs can be derived from exogenous sources as well such as food, alcohol and tobacco [6]. AGEs can be measured invasively in tissues or plasma, or non-invasively using skin autofluorescence (sAF), as some AGEs exhibit fluorescent properties [5, 7–10]. It has been shown that sAF significantly correlates with specific AGEs in skin biopsies [4, 8]. sAF increases physiologically with aging [11, 12]. Its formation increases in the presence of hyperglycemia and oxidative stress [7]. They are therefore assumed to play a major role in the development of cardiometabolic complications [13–17].

Obesity is known to induce systemic oxidative stress (i.e. an imbalance between prooxidants and antioxidants) through multiple biochemical pathways, hyperleptinemia, low antioxidant defense and systemic chronic inflammation among others [18]. As AGEs are prooxidants, it has been assumed that sAF increases more rapidly in subjects with obesity as well [7]. However, studies in adults into the influence of obesity on sAF show conflicting results [3, 4, 19, 20]. No studies measuring sAF have been performed in children/adolescents with obesity, and there are only two studies measuring AGEs in plasma [21, 22].

Therefore, the aim of this study was to evaluate sAF in children with obesity and to compare these results with age-matched lean controls. The results were used to evaluate the association between sAF and metabolic and cardiovascular parameters in children with obesity.

# Materials and methods

For this cross-sectional study into sAF levels of participants with obesity and lean participants, two separate study protocols were used, which were approved by the Medical Ethical Committee of the St. Antonius Hospital, Nieuwegein/Utrecht, The Netherlands (participants with obesity: NL 45664.100.13; lean participants: W14.039). All study procedures were in accordance with the Declaration of Helsinki and the Medical Research Involving Human Subjects Act of the Netherlands. Written informed consent was obtained from the patients and controls and/or parent(s)/caregiver(s).

Participants with obesity (hereafter referred to as patients) were recruited at the pediatric obesity outpatient clinic of the St. Antonius Hospital during the first (intake) visit before intervention. Patients were eligible for inclusion if they were 4–18 years of age and suffered from obesity, defined as a body mass index standard deviation score (BMI-SDS) >2.3 [23, 24]. Patients with type 2 diabetes mellitus (confirmed with an oral glucose tolerance test) were excluded from analysis.

Lean participants (hereafter referred to as controls) were recruited at (high) schools, summer camps and at the pediatric outpatient clinic of the St. Antonius Hospital. Controls were eligible for inclusion if they were 4–18 years of age, had a normal weight defined as BMI-SDS > –1.1 and <1.1 [23, 24] and did not suffer from a somatic disease and/or syndromal disorder.

Information regarding date of birth, date of measurement, sex, skin type, weight, height, smoking habits and alcohol use was collected from both patients and controls, and sAF was measured. In patients, blood pressure and blood samples were additionally collected. In controls, the time of last meal was recorded (<2 h vs. >2 h after meal).

Classification of skin type I–VI was performed by one researcher during the sAF measurement using the Fitzpatrick scale for skin type, which is a classification for human skin color based on skin response to sun exposure (i.e. burns and tans) [25].

Weight was measured to the nearest 0.1 kg and height to the nearest 0.5 cm using calibrated measuring equipment, with participants wearing light clothing and no shoes. BMI was calculated as weight divided by height squared (kg/m$^2$). BMI-SDS and height-SDS were calculated using the Netherlands Organisation for applied scientific research (in Dutch TNO) growth calculator for professionals [26].

Blood pressure was measured in the supine position from the right arm. Hypertension was defined as systolic (SBP) and/or diastolic blood pressure (DBP) ≥95th percentile for age, sex and height. According to the protocol of the pediatric obesity outpatient clinic, blood samples were taken in patients in which hemoglobin $A_{1c}$ ($HbA_{1c}$), fasting plasma glucose (FPG), fasting plasma insulin (FPI), high-density lipoprotein (HDL), low-density protein (LDL), triglycerides and total cholesterol (TC) were measured. Insulin resistance (IR) was calculated using the homeostatic model assessment for insulin resistance (HOMA-IR) (FPG [mmol/L] × FPI [mU/L])/22.5 [27], and defined as HOMA-IR ≥3.4 [28].

sAF was measured using the AGE Reader® (Model "mu"; DiagnOptics Technologies, Groningen, The Netherlands). To perform the measurement, the participant was asked to place the dominant forearm on the AGE Reader® and not to move the arm for approximately 30–60 s. The AGE Reader® illuminated a skin area of 4 cm$^2$ with ultraviolet A light with a single peak excitation wavelength of 370 nm. Emission light (fluorescence in the wavelength of 420–600 nm) and reflected excitation light (with a wavelength of 300–420 nm) from the skin were measured using a spectrometer. sAF was calculated as the ratio between the emission light and reflected excitation light, multiplied by 100 and expressed in arbitrary units (AU) [29].

Participants were stratified by multiple age categories namely: <10, ≥10 to <13, ≥13 to <15, ≥15 to <17, and ≥17 years of age and stratified by skin type I–VI, using the Fitzpatrick scale.

### Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics, version 24 (IBM SPSS Statistics, Chicago, IL, USA). Due to lack of prior data on sAF measurements in pediatric populations with obesity as well as the absence of data on the relationship between sAF and BMI in this population, a formal sample size could not be determined. Therefore, at least two age-matched controls were included for each patient.

Patients were compared with controls using Student's t-test for normally distributed continuous variables and the Mann-Whitney U-test for non-normally distributed data. The chi-squared test was used to analyze differences in categorical variables. When comparing more than two groups, the one-way analysis of variance (ANOVA) or Kruskal-Wallis test for non-parametric data was used.

Correlation analysis between sAF and age, skin type, sex, BMI and cardiometabolic parameters was performed by Spearman's rank or Pearson's correlation in patients. In addition, a multiple linear regression analysis was performed after log transformation to evaluate the influence of obesity on sAF adjusted for skin type, age and sex. An $\alpha$-level of 5% was considered significant for all statistical tests.

# Results

Figure 1 shows the flowchart of the studied population. A total of 143 patients and 428 controls were included. The baseline characteristics are presented in Table 1. Patients and controls were comparable regarding age and sex ($p = 0.82$; $p = 0.98$), but differed significantly regarding skin type, BMI and BMI-SDS (all $p < 0.01$). In patients,

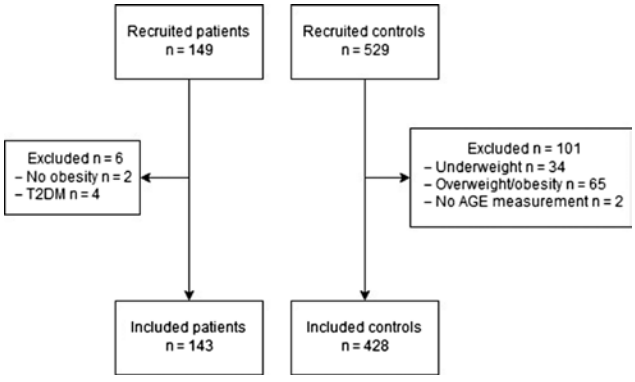


**Figure 1:** Flowchart of the study population.

**Table 1:** Baseline characteristics of patients vs. controls.

| Characteristics | Patients n = 143 | Controls n = 428 | p-Value |
|---|---|---|---|
| Demographics | | | |
| Age, years | 11.8 (3.3–18.4) | 12.2 (4.3–18.8) | 0.82 |
| Sex male, n (%) | 73 (51.0) | 218 (50.9) | 0.98 |
| Skin type, n (%) | | | **<0.01** |
| I | 39 (27.3) | 197 (46.0) | |
| II | 38 (26.6) | 187 (43.7) | |
| III | 37 (25.9) | 18 (4.2) | |
| IV | 23 (16.1) | 18 (4.2) | |
| V | 3 (2.1) | 6 (1.4) | |
| VI | 3 (2.1) | 2 (0.5) | |
| Height, cm | 155.0 (105.0–186.9) | 154.9 (100.5–200.5) | 0.45 |
| Height-SDS | 0.24 (−3.06 to −4.17) | −0.02 (−3.88 to −3.55) | **<0.01** |
| Weight, kg | 64.1 (20.7–154.1) | 41.6 (15.6–84.9) | **<0.01** |
| BMI, kg/m$^2$ | 27.10 (18.52–52.83) | 17.19 (13.96–23.40) | **<0.01** |
| BMI-SDS | 3.22 (2.31–5.72) | 0.04 (−1.10 to −1.10) | **<0.01** |
| Laboratory measurements | | | |
| High SBP, n (%) | 56 (57.3) | NA | |
| High DBP, n (%) | 5 (3.5) | NA | |
| $HbA_{1c}$, mmol/mol | 33 (20–41) | NA | |
| Glucose, mmol/L | 5.2 (4.1–10.3) | NA | |
| Insulin, mmol/L | 14.9 (1.4–96.0) | NA | |
| HOMA-IR | 3.54 (0.30–20.91) | NA | |
| IR, n (%) | 66 (46.2) | NA | |
| Total cholesterol, mmol/L | 4.3 (2.3–7.1) | NA | |
| LDL, mmol/L | 2.7 (1.0–5.0) | NA | |
| HDL, mmol/L | 1.20 (0.58–2.23) | NA | |
| Triglycerides, mmol/L | 1.0 (0.4–2.9) | NA | |

Data are presented as median with range or as frequency with percentage. BMI, body mass index; SDS, standard deviation score; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein. p-Value represents the difference between healthy controls and obesity patients. Bold entries are used for p-values which were below the significance level of <0.05.

the median $HbA_{1c}$ was 33 (20–41) mmol/mol, median HOMA-IR was 3.5 (0.3–20.9) and 66 (46.2%) were classified as IR. None of the patients reported smoking, while three of 428 (0.7%) controls reported smoking. Alcohol consumption on a regular basis was reported by two (1.4%) patients and by none of the controls.

Table 2 shows that sAF in patients was significantly higher compared to controls. This was observed in all age groups except for <10 years of age. When comparing successive age categories in patients, no significant differences between the age groups were observed ($^a p = 0.09$) (Table 2). In controls, sAF was significantly higher in the <10 years of age group in comparison with the ≥10 to <13 and ≥13 to <15 years of age group ($^c p = 0.02$; $^d p = 0.04$), while there was no significant difference compared to the other age categories (Table 2).

Figure 2 shows that sAF differed significantly between skin types in both patients and controls ($p < 0.01$, $p < 0.01$). Post hoc analysis revealed that this difference was due to significantly higher sAF in skin type IV in comparison with type I in patients, and to higher sAF in skin type IV in comparison with I and II in controls (all $p < 0.01$).

As patients differed significantly from controls regarding skin type, a comparison stratified by skin type was performed. As shown in Table 3, no significant differences in sAF were observed between patients and controls for any of the skin types.

No significant sex differences in sAF were observed in patients (males: 1.3 [0.7–2.0] vs. females: 1.3 [0.5–2.4]; $p = 0.94$) or in controls (males: 1.2 [0.5–1.9] vs. females: 1.2 [0.7–2.4]; $p = 0.08$), which is shown in Supplementary Table 1.

No significant difference was observed in sAF regarding the time of last meal (1.2 [0.7–2.4 AU] vs. 1.2 [0.5–2.4 AU]; $p = 0.80$), which is shown in Supplementary Table 2.

In patients, a significant correlation with sAF was observed for skin type ($r = 0.26$; $p = 0.02$), while no significant correlations were observed for age ($r = 0.14$, $p = 0.11$), sex ($r = 0.06$; $p = 0.94$), BMI ($r = 0.14$, $p = 0.01$) or any of the cardiometabolic parameters: SBP ($r = -0.02$; $p = 0.82$), DBP

**Table 2:** sAF levels of patients vs. controls stratified by age category.

| Age category, years | sAF level patients (AU) | sAF level controls (AU) | p-Value |
|---|---|---|---|
| n = 207 | n = 52 | n = 155 | 0.59 |
| **<10** | 1.3 (0.7–2.0) | 1.2 (0.7–1.9) | |
| n = 152 | n = 36 | n = 116 | **0.02** |
| **≥10 to <13** | 1.3 (0.5–2.0) | 1.1 (0.5–2.4)[c] | |
| n = 78 | n = 19 | n = 59 | **0.03** |
| **≥13 to <15** | 1.3 (0.8–1.8) | 1.1 (0.7–1.9)[d] | |
| n = 91 | n = 24 | n = 67 | **0.03** |
| **≥15 to <17** | 1.3 (0.8–2.4) | 1.2 (0.7–2.4) | |
| n = 43 | n = 12 | n = 31 | **<0.01** |
| **≥17** | 1.5 (1.1–1.8) | 1.2 (0.6–1.7) | |
| n = 571 | n = 143 | n = 428 | **<0.01** |
| **All** | 1.3 (0.5–2.4)[a] | 1.2 (0.5–2.4)[b] | |

Data are presented as mean ± SD and median with range. sAF, skin autofluorescence; AU, arbitrary units. p-value represents difference between sAF level of patients and controls. [a]No significant difference in sAF level between patients (p = 0.09). [b]<10 years significantly higher than ≥10 to <13 years (p = 0.02)[c] and ≥13 to <15 years (p = 0.04)[d]. Bold entries are used for p-values which were below the significance level of <0.05.

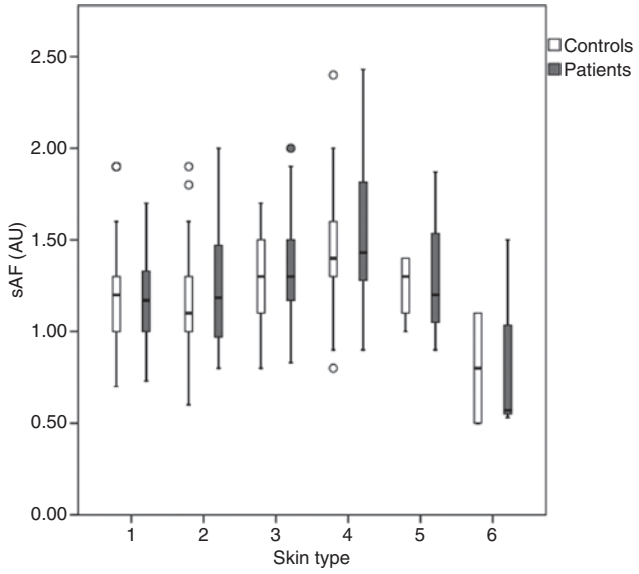


**Figure 2:** sAF levels of patients vs. controls stratified by skin type.

**Table 3:** sAF levels of patients vs. controls stratified by skin type.

| Skin type | sAF level (AU) patients | sAF level (AU) controls | p-Value |
|---|---|---|---|
| n = 236 | n = 39 | n = 197 | 0.99 |
| **I** | 1.2 (0.7–1.7) | 1.2 (0.7–2.4) | |
| n = 225 | n = 38 | n = 187 | 0.40 |
| **II** | 1.2 (0.8–2.0) | 1.1 (0.6–1.9) | |
| n = 55 | n = 37 | n = 18 | 0.54 |
| **III** | 1.3 (0.8–2.0) | 1.3 (0.8–1.7) | |
| n = 41 | n = 23 | n = 18 | 0.58 |
| **IV** | 1.4 (0.9–1.4)[a] | 1.4 (0.8–2.4)[b] | |
| n = 9 | n = 3 | n = 6 | 0.79 |
| **V** | 1.2 (0.9–1.9) | 1.3 (1.0–1.4) | |
| n = 5 | n = 3 | n = 2 | 0.56 |
| **VI** | 0.6 (0.5–1.5) | 0.8 (0.5–1.0) | |

Data are presented as median with range. sAF, skin autofluorescence; AU, arbitrary units. p-Value represents difference between sAF level of obesity patients and normal weight controls. [a]Significantly higher in comparison with type I (p < 0.01). [b]Significantly higher in comparison with type I and II (both p < 0.01). Bold entries are used for p-values which were below the significance level of <0.05.

(r = −0.07; p = 0.44), $HbA_{1c}$ (r = 0.13; p = 0.15), HOMA-IR (r = −0.12, p = 0.16), LDL (r = 0.03; p = 0.97), HDL (r = −0.05; p = 0.56) and triglycerides (r = −0.04; p = 0.65).

In a multivariable regression model, BMI and skin type were statistically significant covariates for sAF (Supplementary Table 3).

## Discussion

Significantly higher sAF was observed in patients compared to controls when stratifying the population by age category, suggesting that obesity is associated with higher sAF. However, this difference between patients and controls was not observed when the population was stratified by skin type. This suggests that sAF in pediatric populations is not (yet) influenced by obesity, but that the difference observed between patients and controls when stratified by age can be explained by the differences in skin type between the groups. However, after adjustment for skin type, age and sex, BMI was a significant covariate for sAF.

This observation is in line with studies in adults in which a correlation between sAF and obesity [4, 19, 20], metabolic syndrome [4, 12, 19] and hypertension [19] was shown. In these studies, significantly higher sAF was observed as well in subjects with obesity [4, 19, 20], metabolic syndrome [4, 12, 19] and hypertension [19]. This is in contrast with the current study in a pediatric population where no difference in sAF was observed, although obesity was a significant covariate as well. It has been suggested that the increase in sAF depends on the duration of obesity and accelerates with longer existing obesity and subsequent development of complications [4]. The difference with the observations in adults might therefore be caused by the fact that our participants were younger and consequently had a shorter duration of obesity. The observation of the current study is in contrast with the results obtained in children with type 1 diabetes mellitus (T1DM), in which no influence of BMI was shown on AGEs measured with skin intrinsic fluorescence (SIF) [30]. The children with T1DM in that study were not diagnosed with obesity (mean BMI-z score 0.6) [30]. As a consequence, AGEs are formed through hyperglycemia and not through oxidative stress which might explain the difference with our study.

In children/adolescents with obesity, only two studies on AGEs have been performed [21, 22]. These studies evaluated AGEs in plasma and showed significant lower plasma AGEs in subjects with obesity compared to controls [21, 22]. These results are in contrast with our observation, and can be explained in our opinion by the difference in the measurement method namely invasive in plasma vs. non-invasive using sAF. Plasma AGEs are not tissue bound and can therefore be excreted by the kidney, the major site of elimination of AGEs. As it is known that renal clearance is higher in populations with obesity, it can be assumed that the clearance of plasma AGEs is higher as well resulting in lower plasma AGEs [4, 21]. Fluorescent AGEs measured with sAF are tissue bound and are not excreted accordingly. Therefore, fluorescent AGEs should give a more appropriate reflection of the total amount of AGEs and thus a better predictor of cardiovascular complications. However, it should be noted that not all AGEs in the skin exhibit fluorescent properties, as a result of which sAF measurements could be an underestimation of the actual AGE content of the skin.

We found significant differences in sAF between the skin types. The highest values were observed in subjects with skin type IV and the lowest with skin type VI, both in controls and patients (Figure 2). It is known that sAF measurements are influenced and impeded in skin type V/VI because dark skin tends to absorb excitation light whereby the sAF levels cannot be reliably measured [10, 29]. This might explain the sudden drop in sAF in subjects with skin types V and VI in our study (Figure 2). It is not completely clear why sAF of subjects with skin type IV was significantly higher compared to those with skin type I–II, as the software of the AGE-reader® has been validated in subjects with skin type I–IV [11, 29]. Previous studies into sAF are often limited by the inclusion of only Caucasian or central Asian subjects who have skin type I–III in general [11, 31, 32]. However, two studies have shown that sAF is influenced by skin type, as subjects with darker skin types had higher sAF compared to subjects with lighter skin types [33, 34]. This was also observed in a study by Felipe et al., measuring AGEs with SIF [30]. Altogether it can be concluded that skin type specific sAF reference values should be used in future research. However, with the possibility of mathematical adjustment of SIF for skin pigmentation eliminating skin type as a confounder, SIF might be a more preferred measurement.

It has been suggested before that AGEs accumulate linearly with age, even in infants [11, 31]. However, in the current study, higher sAF was observed in controls <10 years of age, in comparison with the two subsequent age categories. In our opinion, this might be caused by the technique used by the AGE reader®. For the measurement, a window of 4 $cm^2$ has to be covered by the skin of the individual, which should be possible for young children with a small circumference of the forearm [9, 29]. Nonetheless, it might seem conceivable that ambient light reaches this window along the forearm of a small child, implying more reflectance resulting in misguided higher sAF. This theory may also elucidate why the pattern of sAF difference was not observed in patients with obesity, as it may be assumed that they have a larger circumference of the forearm and consequently cover the window completely. And hereby the non-significant difference in sAF between patients and controls <10 years of age only is explained. It has been described that measurements should be performed in a semi-dark environment to prevent surrounding light from interfering with the measurement [29], although this is not mentioned in the manual of the AGE reader®. Previous studies in children have not described using this technique, but when used, it might clarify the contradiction with our results in controls <10 years [11, 31].

No significant differences in sAF were observed between the other age categories in patients or controls. A possible explanation could be that sAF has not yet varied significantly in children/adolescents, as it takes time for AGEs to accumulate. On the other hand, the annual increase in sAF is suggested to be around 0.023 AU [11, 31]. The defined age categories in our study might therefore

have been too narrow to observe differences in sAF, as the AGE Reader® has an accuracy of 0.1 AU. However, it is questionable whether such small differences in sAF are of clinical relevance. Further research into sAF should therefore use broader age ranges.

To our knowledge, this is the first study on sAF in children/adolescents with obesity. In the current study, the population was divided in stricter age groups than in previous studies on sAF in children/adolescents. In addition, the BMI of the children/adolescents was taken into account. Moreover, data of the patients were compared with age-matched controls. Unfortunately, controls differed significantly from patients regarding skin type, which is caused by the difference in the prevalence of obesity among ethnicities in the Netherlands [35]. As it is known that skin type influences AGEs measured with sAF, a separate analysis for skin type was performed to overcome this issue. However, some limitations should be mentioned. The exact date of diagnosis of obesity and consequently the duration of obesity cannot be defined, as the diagnosis is not based on acute symptoms but on the date that the anthropometric measurements showed a BMI above the cut-off level for obesity. As anthropometric measurements are in general performed at preset intervals, the exact date of diagnosis cannot be defined accurately. Moreover, sAF in controls <10 years of age might have been overestimated due to the interference of surrounding light. In addition, in patients the time of last meal was not recorded and therefore not included in the analysis. As measurements were performed during daytime, when meals contain limited AGEs, the influence of the last meal can be negligible [5, 6]. Moreover, in controls no influence of last meal was noticed. Lastly, only a few controls and patients reported using alcohol or smoking, whereby the influence of these factors could not be taken into account.

## Conclusions

BMI was a covariate for sAF; however, no difference in sAF was observed between children with and without obesity, stratified by skin type. Duration of obesity as well as accuracy of the AGE Reader® might explain this difference. Further research is warranted, in which patients should be matched for age and skin type.

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**Author contributions:** YL recruited participants, collected study data, analyzed the data and wrote the manuscript. LVT recruited participants and critically reviewed and revised the manuscript. MVDV contributed to the conception and design of the study, supervised data collection and critically reviewed and revised the manuscript. CK contributed to the conception and design of the study, critically reviewed and revised the manuscript. MVDV is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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