Skin Autofluorescence, a Measure of Cumulative Metabolic Stress and Advanced Glycation End Products, Decreases During the Summer in Dialysis Patients

*†Bernd Ramsauer, ‡Reindert Graaff, §Aleksandar Sikole, §Lada Trajceska, †Sara Lundström, ¶Stefan Arsov, *Henrik Hadimeri and †Bernd Stegmayr

Abstract: Tissue advanced glycation end products (AGEs) are a measure of cumulative metabolic and oxidative stress and cytokine-driven inflammatory reactions. AGEs are thought to contribute to the cardiovascular complications of hemodialysis (HD) patients. Skin autofluorescence (SAF) is related to the tissue accumulation of AGEs and rises with age. SAF is one of the strongest prognostic markers of mortality in these patients. The content of AGEs is high in barbecue food. Due to the location in northern Sweden, there is a short intense barbecue season between June and August. The aim of this study was to investigate if seasonal variations in SAF exist in HD patients, especially during the barbecue season. SAF was measured noninvasively with an AGE Reader in 34 HD-patients (15 of those with diabetes mellitus, DM). Each time the median of three measures were used. Skin-AF was measured before and after each one HD at the end of February and May in 31 patients (22 men/9 women); the end of May and August in 28 (20 m/8 w); the end of August and March in 25 (19 m/6 w). Paired statistical analyses were performed during all four periods (n = 23, 17 m/6 w); as was HbA1c of those with DM. There was at a median 5.6% increase in skin-AF during the winter period (February–May, P = 0.004) and a 10.6% decrease in the skin-AF during the summer (May–August, P < 0.001). HbA1c in the DM rose during the summer (P = 0.013). In conclusion, skin-AF decreased significantly during the summer. Future studies should look for favorable factors that prevent skin-AF and subsequently cardiovascular diseases. Key Words: Skin autofluorescence—Hemodialysis—Seasonal variations—Advanced glycation end products.
Alzheimer’s disease, diabetic retinopathy, and vascular diabetic complications (10–13).

The skin-AF technique (SAF) is a convenient technique to indirectly measure the burden of AGE and cardiovascular diseases (2). It increases with age and is higher in patients with cardiovascular diseases, diabetes mellitus, and dialysis patients (2,14). A hemodialysis with glucose containing dialysate reduces plasma but not skin-AF levels (15) independent of dialyzer used (16) while a glucose-free dialysate improves skin-AF (17). The extent of annual increase is an even further prognostic factor (18,19). Data of a direct coupling to nutritional factors were not described (20).

It is known that fried and grilled foods contribute to high dietary intake of AGEs (21–24). Barbecuing is popular in Sweden during the summer season, between mid of June and August (25).

The aim of this study was to investigate if a seasonal variation in SAF exists in HD patients, especially during the barbecue season.

PATIENTS AND METHODS

Study design

A longitudinal observation study was performed at the HD center at the University Hospital in Umeå, in northern Sweden.

Patients at the HD ward were consecutively informed and included in the study. Excluded were eight patients: three with active inflammatory disease, two unable to give consent, two refused to participate in the study, and one patient was excluded since he performed home HD and was not able to show up for skin-AF measures. All patients were on chronic HD. Each time the median value of three SAF measurements (taken at different locations on the lower arm) were used. Skin-AF measurements were made at four time points: the ends of February (T1), May (T2), August (T3), and March (T4; 13 month after the first measurement). Patients were asked to fill out a questionnaire of their food habits before start of the barbecue season (May T2) and after the end of the summer period (August T3).

During the study period 34 patients on chronic HD were included (24 male/10 female). The median age at T2 was 68.5 years (range 33–83). The median vintage of HD was 23.5 months (range 3–109 months). The main reasons for HD were primary glomerular disorders for eight patients, diabetic nephropathy for nine patients (3 with diabetes mellitus type 1, and 6 with type 2), polycystic kidney disease in 3, hypertension and/or renovascular disease for eight patients, and other or unknown diagnosis for the remaining six patients (Table 1). Besides renal diagnoses 15 also suffered from diabetes mellitus.

The number of patients varied between the different periods, because some of the patients ended HD due to death or transplantation, while others entered the dialysis program during the evolving study period. Paired statistical comparison was made in 31 patients (22 men, 9 women) during the first period (T1 to T2), 28 patients (20 men, 8 women) between T2 and T3, and 25 patients (19 men, 6 women) between T3 and T4. This means that data of each patient were analyzed as its own control. Thereby, data at the first occasion were compared with data at the subsequent occasions. Therefore, inter-individual differences could be expected during the investigation.

The weekly dialysis performance is given as Kt/V (calculated weekly removal of urea by HD). Numerous patients who enter the HD program still have some residual renal function. Therefore, in their early phase of dialysis, they performed only two dialyses/week and less hours than when residual renal function totally ceased. Based on decline in residual renal function, the extent of prescribed hours on dialysis expanded over time, as well as dialysis days per week. This measure is to maintain adequate overall clearance (residual renal clearance + clearance performed by dialysis). This will result in a reduction of residual renal function while Kt/V achieved by dialysis rises to a certain extent.

Twenty-three of the patients were included in the study at all four points in time. Current tobacco users were too few for statistical comparisons (1 cigarette smoker and 2 snuffers).

Ethical considerations

All patients included were informed and gave their consent. The local ethics committee approved the study (Dnr 08-023M).

AGE reader

The AGE Reader (Diagnoptics Technologies BV, Groningen, The Netherlands) illuminates a skin surface (usually lower arm) of ~4 cm², that is guarded against surrounding light, and is equipped with a light source that provides UV-A light between 350 and 420 nm (peak wavelength 370 nm). Reflected light from the skin and autofluorescence are measured simultaneously, in a semi-dark environment, using a spectrometer within the instrument. The skin-AF was measured in arbitrary units (AU). The arbitrary unit was based on the ratio of the average
light intensity per nanometer in the range between 420 and 600 nm and the average light intensity per nm in the range between 300 and 420 nm. Version 2.3 of the AGE Reader software was used. The AGE Reader has been validated and more extensively described in previous studies (2,3,26). Those authors compared data from SAF and skin biopsies taken from the same site of the arm. Those biopsies were analyzed for collagen-linked fluorescence, for pentosidine (a fluorescent AGE), N-ε-carboxy-methyl-lysine (CML) and N-ε-(carboxyethyl)-lysine (CEL, nonfluorescent AGEs). They found significant correlations between SAF and pentosidine and collagen-linked fluorescence, as well as for CML and CEL (2,3). Skin-AF, before and after the HD, were obtained as a median of three measurements each time along the same forearm at slightly different positions in a semi-dark environment at room temperature on each occasion. The value of SAF before and after HD was applied for further analysis. If only one sample was achieved (either before or after the same dialysis) the single value was representing both occasions since previous studies showed no difference in SAF before versus after standard HD (15),(16).

Conditions were kept similar throughout the study: glucose containing dialysate (5 mmol/L in final concentration), dialysate flow 500 mL/min, including patients using the same dialyzers and dialysis technique. Dialyzers used were from Fresenius Medical Care (Bad Homburg, Germany; FX10 or FX80 dialyzers, both with a surface area of 1.8 sqm) and used in 2 versus 12 patients and Gambro dialyzers (yF17L, yF140H and yF210H; the surface areas were 1.7 , 1.4 and 2.1 sqm, respectively) used in 1, 6 and 10 patients, respectively. Data of dialyzers were missing in three patients. Blood flow was kept similar for each patient and was based on the function of the A V-fistula, AV-graft or central dialysis catheter (CDC). Available data for 17 patients showed that they performed HD with a median blood flow of 290 mL/min (range 190–380 mL/min).

The variables checked for were urea reduction rate (URR), Kt/V, and 25(lH) vitamin D levels in serum that were examined before and after the summer period (T2 versus T3). The weekly dose of the alfa-calcidol (1α-(OH)D3 vitamin) was registered. The metabolic control of patients with diabetes mellitus was during the period performed with the HbA1c Mono 1 method (reference value 3.6–5.0 %).

**Food intake**

Questionnaires were distributed to reveal intake of food such as potatoes, pasta, rice, sausage, fish,
Statistical analysis

SPSS statistical software (IBM SPSS Statistics 20.0) was used for the analyses. Power calculations were performed using G*Power (V3.1.9.2 or Windows) (27). Since most patients were assumed to be exposed to barbecued food, the expected effect size was set to more than 0.65 and the power at 0.8. The calculated sample size necessary for paired statistical analysis (two-tailed analysis) was 22 or more pairs. To avoid an effect of nonnormal distributed data, we use nonparametric paired statistics when we compare changes over time. The ‘pair’ means that we compare each individual to himself/herself to avoid inter-individual interference. Paired statistical analysis was made by Wilcoxon test and for independent group comparison Mann–Whitney U-test was used (for nonnormally distributed data). Median values and range are given for nonparametric calculations, and mean values ± standard deviation for parametric calculations. For correlations the Spearman’s rank correlation coefficient was used. A two-tailed P value of less than 0.05 was considered significant. Dialysis skin AF values were calculated as the mean of skin-AF measured before HD and after HD. Multiple regression analyses (stepwise) were performed with either skin-AF at T3 as a dependent factor or the change in skin-AF from T2 to T3 as a dependent factor. Analysis was also performed for persons who had fulfilled measurements at all four time points. Variables investigated in the model were age, gender, diabetes mellitus, tobacco habits, HD vintage, dialysis dose (hours/session and hours/week), parathyroid hormone (PTH) levels, change in 25(OH) vitamin D levels from May to August, medication such as dose of alfa-calcidol (1α(OH)D3 vitamin), use of beta-blockers, calcium channel blockers, ACE inhibitors, angiotensin receptor blockers or statins. The IBM SPSS software (version 22) was used.

RESULTS

The comorbidity of the patients included hypertension (n = 29, 85%), CVD (n = 18, 53%), and among patients with CVD eight suffered from previous myocardial infarction (23.5%) and four from strokes (12%). Lifestyle factors included tobacco use either previously (62%, missing data in 2), current (8.8%) or never users (32.4%) and alcohol consumption (wine and beer 35.3%, data missing in 3). All patients were on chronic HD with the median treatment duration/session of 4 h (range 3–5 h). Median value of Kt/V was 1.39 (0.82–2.12) and median of urea reduction rate (URR) was 71% (55–83%) (Table 1). When comparing Kt/V from patients included through the whole period there was no difference in URR and Kt/V during the seasonal periods. In these patients, the mean initial value of Kt/V was 1.41 (±0.40) with a nonsignificant increase of +3.0% until T2, another +2.9% at T3 and +7.0% at T4. The Kt/V increase (based on mean values) over the whole period was 13.4% (P = 0.004). The urine volume was measured in February and August, year 1. Oliguria or anuria (500 mL or less) was present in 13 patients. In the 16 other patients where paired data were available, the volume was nonsignificantly changed from a mean of 1274 ± 764 to 1220 ± 660 mL/24 h in August. Five patients performed low flux dialyses through the period, 20 performed high flux HD and 6 hemodi-afiltration. There was no difference in SAF values at start or end of dialysis or in the change in SAF in any of the dialysis types, except that the SAF values after HD measured in August were significantly lower for those with low flux HD versus those with high flux HD (P = 0.027).

There was a significant increase in skin-AF by a median of 5.6% between the period T1 and T2 (P = 0.004). The median skin-AF decreased by 10.6% (P < 0.001) during the 3 month summer period (T2 until T3). There was a nonsignificant increase of 4.2% in skin-AF between T3 and T4. There was a nonsignificant increase of 2.1% in skin-AF from T1 to T4, that is, 13 months later (Table 2). When only analyzing patients who performed all four measurements (at T1, T2, T3 and T4, n = 23), the results were similar to the groups above (Table 3).

SAF measured in women was significantly lower than in men at all periods. The change in SAF during the periods were similar in men and women (Table 4).

There was no correlation between the change in skin-AF (T2 to T3) and the baseline levels or in the change in 25(OH) vitamin D level during the summer. The change in skin-AF (ΔT2–T3) was not related to URR or Kt/V as estimates of dialysis efficacy. Previous smokers (n = 17) versus never smokers (n = 12) did not differ with regard to baseline skin-AF at T2 or T3 nor in the change of skin-AF.

In univariate analysis, skin-AF did not correlate with age, HD vintage, URR, Kt/V, PTH levels, 25(OH) vitamin D levels nor to the medication such as dose of alfa-calcidol (1α(OH)D3 vitamin) or the use of beta-blockers, calcium channel blockers, ACE inhibitors, angiotensin receptor blockers or statins.
the group that was followed over the whole period (n = 23) the plasma level of 25(OH) vitamin D increased during the summer (P = 0.048) and decreased thereafter toward March next year (P = 0.002).

In a multi-variate regression analysis, there was a significance of the model (r² 0.514, P = 0.001). The remaining factors for high SAF at T3 were male gender (P = 0.008) and a lower increase of 25(OH) vitamin D levels during the summer period (P = 0.033).

Vitamin D administration was by alfa-calcidol (1α-(OH)D3 vitamin), that was mainly kept unchanged during the study period.

HbA1c was analyzed over time, in patients with diabetes mellitus (n = 15, Mean ± SD values at T1: 5.6 ± 1.7, T2: 6.2 ± 2.2, T3: 7.0 ± 2.1, T4: 5.9 ± 2.1%). Paired analyses revealed a significant increase from May until August (P = 0.013), and a nonsignificant reduction from August to March (P = 0.054). In patients with diabetes mellitus (n = 12) during the period May and August SAF was significantly reduced, both the predialysis as well as the postdialysis values (P = 0.005 versus P = 0.016). The change in HbA1c was not correlated to the change in SAF during the period May to August (n = 10 DM patients with both data).

Questionnaires about diet intake were filled out by 15 of the 28 (54%) before the barbecue season and 7 of 28 (25%) after the season. Univariate correlation analyses between SAF value in May (T2) and the intake of French fries was significantly inverse (rₒ = −0.74, P = 0.002, n = 15) while no other diet variable showed correlation. French fries, but not SAF, correlated with intake of pasta (rₒ = 0.54, P = 0.038, n = 15), grilled meat (rₒ = 0.53, P = 0.041, n = 15) and hamburgers (rₒ = 0.67, P = 0.007, n = 15). Seven of the patients responded after the season (all pairs). There was no significant difference in intake of grilled food in that period.

### Table 2. Mean, median and change of SAF between the different periods

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>31</td>
<td>3.87</td>
<td>±0.61</td>
<td>3.97</td>
<td>1.09</td>
</tr>
<tr>
<td>T2</td>
<td>31</td>
<td>4.10</td>
<td>±0.69</td>
<td>4.00</td>
<td>1.03</td>
</tr>
<tr>
<td>ΔT2–T1</td>
<td>31</td>
<td>0.22</td>
<td>±0.40</td>
<td>0.24</td>
<td>0.49</td>
</tr>
<tr>
<td>Δ% T2–T1</td>
<td>31</td>
<td>0.061</td>
<td>±0.11</td>
<td>0.056</td>
<td>0.14</td>
</tr>
<tr>
<td>T2</td>
<td>28</td>
<td>4.08</td>
<td>±0.67</td>
<td>3.99</td>
<td>0.95</td>
</tr>
<tr>
<td>T3</td>
<td>28</td>
<td>3.67</td>
<td>±0.81</td>
<td>3.63</td>
<td>1.17</td>
</tr>
<tr>
<td>ΔT3–T2</td>
<td>28</td>
<td>−0.41</td>
<td>±0.43</td>
<td>−0.43</td>
<td>0.45</td>
</tr>
<tr>
<td>Δ% T3–T2</td>
<td>28</td>
<td>−0.104</td>
<td>±0.12</td>
<td>−0.106</td>
<td>0.11</td>
</tr>
<tr>
<td>T3</td>
<td>25</td>
<td>3.75</td>
<td>±0.78</td>
<td>3.63</td>
<td>1.03</td>
</tr>
<tr>
<td>T4</td>
<td>25</td>
<td>3.93</td>
<td>±0.68</td>
<td>4.01</td>
<td>0.68</td>
</tr>
<tr>
<td>ΔT4–T3</td>
<td>25</td>
<td>0.18</td>
<td>±0.47</td>
<td>0.17</td>
<td>0.71</td>
</tr>
<tr>
<td>Δ% T4–T3</td>
<td>25</td>
<td>0.07</td>
<td>±0.17</td>
<td>0.042</td>
<td>0.18</td>
</tr>
</tbody>
</table>

SD = standard deviation, IQR = interquartile range, T1 = February, T2 = May, T3 = August, T4 = March (13 month after T1).

### Table 3. Data included only from patients that had data available at all points of time. Mean, median and the change of SAF between the different periods

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>23</td>
<td>3.88</td>
<td>±0.62</td>
<td>3.95</td>
<td>1.13</td>
</tr>
<tr>
<td>T2</td>
<td>23</td>
<td>4.15</td>
<td>±0.68</td>
<td>4.02</td>
<td>0.85</td>
</tr>
<tr>
<td>T3</td>
<td>23</td>
<td>3.77</td>
<td>±0.82</td>
<td>3.87</td>
<td>1.16</td>
</tr>
<tr>
<td>T4</td>
<td>23</td>
<td>3.93</td>
<td>±0.71</td>
<td>4.03</td>
<td>0.74</td>
</tr>
<tr>
<td>ΔT2–T1</td>
<td>23</td>
<td>0.27</td>
<td>±0.42</td>
<td>0.33</td>
<td>0.49</td>
</tr>
<tr>
<td>Δ% T2–T1</td>
<td>23</td>
<td>0.07</td>
<td>±0.10</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>ΔT3–T2</td>
<td>23</td>
<td>−0.38</td>
<td>±0.45</td>
<td>−0.43</td>
<td>0.45</td>
</tr>
<tr>
<td>Δ% T3–T2</td>
<td>23</td>
<td>−0.09</td>
<td>±0.13</td>
<td>−0.10</td>
<td>0.13</td>
</tr>
<tr>
<td>ΔT4–T3</td>
<td>23</td>
<td>0.16</td>
<td>±0.48</td>
<td>0.01</td>
<td>0.48</td>
</tr>
<tr>
<td>Δ% T4–T3</td>
<td>23</td>
<td>0.07</td>
<td>±0.17</td>
<td>0.0</td>
<td>0.17</td>
</tr>
</tbody>
</table>

SD = standard deviation, IQR = interquartile range, T1 = February, T2 = May, T3 = August, T4 = March (13 months after T1).
DISCUSSION

This study extends previous studies using SAF as a tool for measurement of retained glucose degradation products (3,26).

Since SAF is a strong marker for the presence and risk of CVD as well as of mortality (2), it is important to halt the rate of increase and if possible even reverse it.

The present study showed a significant decrease of more than 10% in SAF during the summer period in contrast to an increase of 5% during the period before that. These differences were not related to specific differences in dialysis efficacy. The importance of the reduction of SAF during the summer season seems evident when comparing the present study with the increase in SAF noted during the first period (February–May) of 0.22 unit/3 months (approximately 0.88 AU per year). This is much more than the increase per year found in Macedonian patients with chronic HD with diabetes mellitus and cardiovascular disease (19) that had an increase in SAF of 0.34 AU per year.

Since only few questionnaires helped to estimate food habits during the summer period, the increased HbA1c during the summer, found in patients with DM, indicated also increased intake of food that might interfere with glycation of proteins. However, in contrast to an expected further increase in SAF by more ingested food during the barbecue season (21,22,25), there was a reduction in SAF during the summer. Under normal conditions AGEs are more rapidly formed during oxidative stress, and several of the end products of glyoxidation accumulate in tissue that results in cross-links with proteins, inducing tissue stiffening of blood vessels and skin and subsequent atherosclerotic lesions (78). We did not investigate oxidative stress in the present study. However, our data do not rule out beneficial effects during the summer period which may enable a recovery of tissue with less residual accumulated AGEs. In the DM patients, the contrast of having increased HbA1c and in parallel significantly reduced SAF is notable. HbA1c as a marker of glycation of hemoglobin is located within the vessels. An increased level of HbA1c therefore is a more direct indicator of glucose metabolism and burden in the vessels. However, SAF is a marker for more extravascular AGE burden. It may well be possible that these mechanisms do not act in parallel, and no correlation indicated such physiology.

In a previous study, caloric protein and AGE intake hardly influenced the one-year ΔAF in HD patients (28). The SAF reduction indicates that other factors, independent of nutrition, may have caused the decrease of SAF during the summer period. Such beneficial effect could be due to the more intensive insolation during the summer and the subsequent activation of the cholecalciferol synthesis. In vitro studies have supported calcitriol or 1,25(OH)2D as a vascular protective agent by showing that it reduces the deleterious effect of AGEs on the endothelium (29). Vitamin D has been found to reduce the deposits of AGEs in the aortic wall and even reduce the systemic oxidative stress in diabetic rats (30). Those data are supported by another study where a lower serum 25(OH)D was associated with higher
skin-AF (31). However, this may not be true for the HD population, as the conversion from Vitamin D to 1,25(OH)2D by the kidneys is expected to be very limited in these subjects. Therefore, it is possible that also extra-renal conversion of 25(OH)D into 1,25(OH)2D plays a role (32). If this factor is of importance, an option would be to apply additional supplementation of Vitamin D until a sufficient level of 25(OH)D is reached, and/or to increase the level of 1,25(OH)2D by alfa-calcidol (1α-(OH)D3 vitamin) that is effectively converted by the liver. However, the doses of alfa-calcidol administered to the patients within the frame of the present study did not show any correlations to the change in SAF. Beneficial effects of sun exposure could be strengthened by a Swedish study where they found that sun exposure was inversely related to cardiovascular diseases and all-cause mortality in a ‘dose dependent’ manner (33). They even found that avoidance of sun exposure was a risk factor for all-cause mortality. Notably, in northern Sweden the summer season is short and this may be a reason that differences between nonexposure and exposure to sun are more extensive than in areas closer to the equator. The present study indicates that future studies should investigate if factors such as 25(OH)D(3) may prevent AGEs to deposit in tissue and also possible effects of diets.

Although the dietary questionnaires were limited in numbers, a strong significant inverse correlation existed between the SAF value and the intake of French fries. An assumption, for future studies, could be that the data may be due to the fact that French fries contain a considerable amount of nonsaturated fat (34) that may have had beneficial effect in limiting the development of SAF. This type of fat would be in line with Mediterranean living and diet, considered as being more healthy (35). However, due to poor compliance with response to dietary questionnaires, including only 25% of patients, we prefer not to draw firm conclusions of those results.

CONCLUSION

This study showed unexpected reduction in skin autofluorescence in hemodialysis patients during the summer period. The possible beneficial effect of sun illumination should be further investigated together with factors such as diet and vitamin D supplementation.

Acknowledgments: Funding was supported by the County Council Vasterbotten (Central ALF, project: 7001586), Sweden, Njurföreningen Västerbotten, Sweden, and Skaraborgs Hospital (FoU), Sweden. Thanks to professor Ingegerd Johansson, Umeå University for recommendations regarding the dietary questionnaires.

Authors Contributions: The various contributions to the study were by design (BeS, RG, AS), applications (BeS), clinical fulfillment (BeS), data cache (BeS, RG, SA), collection and preparation of data (BR, RG, BeS), dietary questionnaire (SL), statistical analyses (BR, RG, BeS), manuscript preparation primarily (BR) and subsequently (BR, BeS, RG, HH, SA, LT) and supervision of PhD student BR (BeS, HH).

Conflict of Interest: RG is founder and stakeholder of Diagnoptics Technologies, the manufacturer of the AGE Reader. None of the other authors reported a conflict of interest with the outcome of the study.

REFERENCES


