Dermato-Endocrinology 4:1, 33–38; January/February/March 2012; © 2012 Landes Bioscience

Advanced glycation end products measured by skin autofluorescence in a population with central obesity

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Keywords: skin autofluorescence, advanced glycation endproducts, central obesity

Abbreviations: AGEs, advanced glycation end products; skin AF, skin autofluorescence

Accumulation of advanced glycation end products (AGEs) is enhanced by chronic hyperglycemia and oxidative stress and this process may contribute to the pathogenesis of vascular disease. Skin autofluorescence (AF), a measure of accumulation of AGEs in skin collagen, is associated with vascular disease in patients with diabetes.

Because central obesity enhances oxidative stress people with central obesity might already have increased accumulation of AGEs before diabetes or cardiovascular disease become manifest. To test this hypothesis, we compared the distribution of skin AF and its association with clinical and biochemical parameters in individuals with and without central obesity.

Skin AF was measured by a validated AGE Reader in 816 persons with and 431 persons without central obesity, aged 20–70 y.

Mean skin AF increased with age and smoking and was higher in centrally obese individuals compared with non-obese individuals ($p = 0.001$, after adjustment for age and smoking $p = 0.13$). Mean skin AF in the subgroups without central obesity and without other risk factors ($n = 106$), central obesity without other risk factors ($n = 74$) and central obesity with other risk factors ($n = 742$) was 1.63 ± 0.37, 1.74 ± 0.44 and 1.87 ± 0.43 AU, respectively (p for trend < 0.001, after adjustment for age and smoking p for trend $= 0.12$).

In the group with central obesity age, current smoking, alcohol consumption, waist circumference, creatinine clearance and hs-CRP were independently associated with skin AF ($R^2 = 29.4\%$). Waist circumference hardly contributed to the explained variance. The relationship between waist circumference and skin AF is not as obvious as we hypothesized.

Introduction

Advanced glycation end products (AGEs) are modifications of proteins, lipids or nucleic acids that become non-enzymatically glycated and oxidized after contact with reducing sugars. This process may contribute to the pathogenesis of micro- and macrovascular disease.[1] In diabetes, accumulation of AGEs in skin collagen is correlated with the presence of long-term vascular complications.[2]

AGEs accumulate with aging in normal condition. The formation and accumulation of AGEs is enhanced by chronic hyperglycemia, oxidative stress and a decreased renal function. AGEs can also be derived from exogenous sources such as tobacco smoke and food.[3,4]

Some AGEs, such as pentosidine, exhibit autofluorescence. An AGE Reader uses this property and measures skin autofluorescence (AF) non-invasively and within seconds. Skin AF as measured by an AGE Reader significantly correlates with collagen-linked fluorescence and with specific AGEs such as pentosidine, N^{ε}-(carboxymethyl)-lysine (CML) and N^{ε}-(carboxyethyl)-lysine (CEL) in skin biopsies.[2]

The relationship between skin AF and micro- and macrovascular disease has been studied in patients with diabetes, renal failure and cardiac disease.[5-8] Skin AF was shown to be associated with micro- and macrovascular complications in type 2 diabetes and to be an independent predictor of cardiovascular morbidity and mortality in patients with type 2 diabetes or cardiac disease.

People with central obesity have an up to 5-fold increased risk for developing diabetes. Waist circumference predicts the likelihood of developing diabetes beyond that explained by commonly evaluated cardiometabolic risk factors and body mass index (BMI).[9-11] Central obesity enhances oxidative stress, also in non-diabetic subjects.[12-14] Oxidative stress leads to insulin resistance and β-cell dysfunction, and this in turn can increase oxidative stress, accelerating the progression to overt type 2 diabetes and diabetes related complications.[15-17] Taking this into

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Submitted: 06/09/11; Revised: 08/25/11; Accepted: 09/06/11
http://dx.doi.org/10.4161/derm.17999

account, people with central obesity might already have increased levels of AGEs before diabetes or cardiovascular disease become manifest.

Skin AF is, to the best of our knowledge, not assessed in a population with central obesity. To evaluate our hypothesis of accelerated accumulation of AGEs in central obesity, we compared the distribution of skin AF as well as the associations between skin AF and several clinical and biochemical parameters in people not treated for diabetes, hypertension or lipid disorders, with and without central obesity.

Results

In total 1,860 participants underwent all screening procedures, in 1,386 of them skin AF and reflectance were measured. 1,247 (90.0%) were Western European, with a skin reflection of at least 6.0%. Their mean age was 45.0 y and 65.7% was female. 330 of them fulfilled the NCEP ATP III metabolic syndrome criteria.

Mean skin AF in the total population was 1.83 ± 0.42 AU. For the ten year age categories 20–29 y, 30–39 y, 40–49 y, 50–59 y and 60–69 y, mean skin AF was 1.39 ± 0.22, 1.59 ± 0.31, 1.84 ± 0.37, 2.03 ± 0.41 and 2.21 ± 0.47 AU, respectively.

Eight-hundred sixteen of the 1,247 persons had a waist circumference above 88 /102 cm (women / men); 431 had a waist circumference equal to or below this threshold. In both groups, smokers had a 12% respectively 17% higher skin AF than non-smokers (2.03 ± 0.48 vs. 1.82 ± 0.41 AU; $p < 0.001$ and 2.01 ± 0.48 vs. 1.72 ± 0.36 AU; $p < 0.001$).

Table 1 shows the characteristics of both participants with and without central obesity. Blood pressure, blood lipids, fasting glucose and hs-CRP differed across groups. Mean skin AF in the centrally obese participants was 1.86 ± 0.43 AU, compared with 1.78 ± 0.40 AU in the group without central obesity (p = 0.001, after adjustment for age and smoking p = 0.13). Mean skin AF in the groups without central obesity and without other risk factors (n = 106), central obesity without other risk factors (n = 74) and central obesity with other risk factors (n = 742) were 1.63 ± 0.37, 1.74 ± 0.44 and 1.87 ± 0.43 AU, respectively (p for trend < 0.001, after adjustment for age and smoking p for trend = 0.12).

Participants with the metabolic syndrome (n = 330) had a higher skin AF than participants without the metabolic syndrome (1.90 ± 0.43 vs. 1.81 ± 0.42 AU; p = 0.001). Also within the centrally obese participants (n = 816), those with the metabolic syndrome (n = 319) had a higher skin AF than those without (1.90 ± 0.43 vs. 1.84 ± 0.44 AU; p = 0.07).

Table 1. Patient characteristics for individuals with and without central obesity

Characteristics	Central obesity n = 816	No central obesity n = 431	p value
Age (years)	45.8 ± 9.4	43.6 ± 10.0	< 0.001
Gender (female)	67.2%	62.9%	0.13
Waist circumference female (cm)	98.4 ± 8.8	82.0 ± 5.1	< 0.001
Waist circumference male (cm)	109.9 ± 6.7	96.0 ± 6.3	< 0.001
BMI (kg/m^2)	29.8 ± 3.9	24.8 ± 2.4	< 0.001
Systolic blood pressure (mmHg)	138.1 ± 17.0	129.0 ± 15.6	< 0.001
Diastolic blood pressure (mmHg)	85.2 ± 9.2	80.4 ± 9.7	< 0.001
Triglycerides (mmol/L)	1.5 ± 1.0	1.1 ± 0.7	< 0.001
HDL-cholesterol (mmol/L)	1.5 ± 0.4	1.6 ± 0.4	< 0.001
LDL-cholesterol (mmol/L)	3.1 ± 0.9	3.0 ± 0.9	< 0.01
Fasting glucose (mmol/L)	5.0 ± 1.0	4.8 ± 0.6	< 0.001
Hs-CRP (mg/L)	1.8 (0.5)	1.7 (0.5)	< 0.001
Albumin to creatinine ratio (mg/mmol)	0.9 ± 2.5	0.7 ± 1.1	0.04
Creatinine clearance (mL/s)	2.6 ± 0.6	2.2 ± 0.5	< 0.001
Physical activity > 150 min/wk	55.3%	59.4%	0.16
Current tobacco smoking	20.1%	18.6%	0.51
Family history of diabetes	15.6%	11.8%	0.07
Current alcohol consumption none moderate excessive	 25.8% 66.7% 7.5%	 21.9% 72.7% 5.4%	0.08
Skin AF (AU)	1.86 ± 0.43	1.78 ± 0.40	< 0.01
Skin reflectance (%)	20.6 ± 7.1	18.6 ± 6.2	< 0.001

Normally distributed variables are expressed as mean ± SD. Not normally distributed variables are expressed as median (interquartile range). Nominal variables are expressed as percentages.

In univariable regression analysis in the 816 individuals with central obesity age, waist circumference in women, systolic and diastolic blood pressure, HDL-cholesterol, LDL-cholesterol, fasting glucose, hs-CRP, creatinine clearance and smoking showed a significant association with skin AF. In individuals without central obesity diastolic blood pressure and HDL-cholesterol did not show a significant association with skin AF; waist circumference was only significant in men. The other associations were similar across people with and without central obesity.

Table 2 shows the multivariable associations between skin AF and the clinical and biochemical parameters for individuals with and without central obesity. In people with central obesity, age, current tobacco smoking, moderate alcohol consumption, creatinine clearance, hs-CRP and waist circumference were independently associated with skin AF. Together these variables explained 29.4% of the total variance of skin AF. Age and current tobacco smoking together explained 26.9% of the total variance. Adding waist circumference to the limited model with only age and current tobacco smoking led to an increase in explained variance from 26.9% to 27.5%. In the subgroup of 74 centrally obese individuals without other cardiometabolic risk factors the variance explained by age and smoking was 29.2%. None of the other variables showed an independent association with skin AF in this group. In the population without central obesity not only age, current tobacco smoking and alcohol consumption were independently associated with skin AF, but also a family history of diabetes. These variables together explained 39.1% of the total variance. Age and current tobacco smoking together explained 36.4% of the total variance. However, in people without central obesity creatinine clearance and hs-CRP were not associated with skin AF.

Discussion

This study shows that mean skin AF for a Western European population with central obesity but without previously known cardiovascular risk factors, aged 20–70, does not differ significantly between centrally obese and non-obese people, after adjustment for age and smoking. Age, current tobacco smoking, current alcohol consumption, waist circumference, creatinine clearance and hs-CRP were independently associated with skin AF and together these variables explained 29.4% of the total variance.

Mean skin AF in our apparently healthy study population, in both centrally obese and non-obese persons, was lower than skin AF in control groups of other studies. Lutgers et al. measured skin AF in non-diabetic pre-operative visitors of an out-patient clinic. They were both obese and non-obese, with a mean age of 52 ± 17 y (n = 231). The average skin AF in this group was 2.14 ± 0.6 AU.[6] This is higher than mean skin AF in our population, which might in part be explained by the lower mean age in our study. When comparing skin AF within similar age categories, the mean values for the people aged 40–49 y were comparable. However, in the age groups 50–59 and 60–69 y, the mean values in our study are respectively 4% and 11% lower in centrally obese people and even more in non-obese people. In addition, Koetsier et al. provide reference values of skin AF for the age groups 20–29 and 30–39 y.[18] These values are based on the combined data of the cohort described by Lutgers et al. and another cohort of patients without known history of hypertension, diabetes, or cardiovascular disease. Compared with these age groups, the mean values are respectively 7% and 8% lower in our centrally obese group and even more in the non-obese group. Possible explanations for these differences are the lower percentage of current smokers in our population compared with the population described by Lutgers et al. (20% vs. 37%). Also the measurement of skin AF after an overnight fast as performed in our study could contribute. Stirban et al. have shown that postprandial both vasodilatation of the microcirculation and an acute increase in serum AGEs occur. They suggest this could explain the increase in skin AF—about 9% in healthy persons—that they found postprandially.[19]

We found six variables to be independently associated with skin AF in a centrally obese population: age, current tobacco smoking, alcohol consumption, waist circumference, creatinine clearance

Table 2. Determinants of skin autofluorescence in multivariable linear regression analysis for individuals with and without central obesity

Characteristics	Central obesity n = 816		No central obesity n = 431	
	R^2 29.4%		R^2 39.1%	
	B	95% CI	B	95% CI
Age (years)	0.019	0.016; 0.023	0.023	0.020; 0.026
Waist circumference (cm)	0.005	0.001; 0.009		
Hs-CRP (mg/L)	0.028	0.001; 0.055		
Creatinine clearance (mL/s)	-0.001	-0.002; -0.000		
Current tobacco smoking	0.201	0.137; 0.264	0.282	0.204; 0.359
Family history of diabetes			0.122	0.028; 0.215
Current alcohol consumption				
none				
moderate	-0.093	-0.152; -0.034	-0.094	-0.168; -0.021
heavy	-0.037	-0.144; 0.070	-0.161	-0.309; -0.013

and hs-CRP. Age and current tobacco smoking are known to be important factors in determining skin AF in healthy individuals.[6] The negative association between creatinine clearance and skin AF can be explained from the fact that the kidney is the major site of AGE clearance.[3] The relationship between current alcohol consumption and skin AF was not assessed before. Moderate alcohol consumption seems to be independently associated with a lower skin AF. This is in accordance with the fact that moderate alcohol intake has beneficial effects on endothelial function, reduces inflammation and thereby lowers cardiovascular risk.[20,21]

Lutgers et al. were the only ones to look at the relationship between obesity and skin AF in a population without established diabetes or cardiovascular disease. In their multivariable analysis BMI, as measure of obesity, did not show a significant association with skin AF. Findings in populations with type 2 diabetes are inconsistent. Both Lutgers et al. and Meerwaldt et al. reported significant univariable associations between BMI and skin AF; however in multivariable analyses these associations lost their significance.[6,8] Monami et al. did find significant correlations for both WC and BMI after correction for age, sex and HbA1c.[22] The significant (though limited) independent association we observed in our centrally obese population supports our hypothesis that people with central obesity already have increased levels of AGEs before diabetes or cardiovascular disease become manifest. This is further supported by the trend in increasing skin AF when looking successively at subgroups of healthy non-obese, healthy obese and unhealthy obese groups. Perhaps with a longer existing central obesity state and the subsequent development of other cardiometabolic risk factors the accumulation of AGEs accelerates, leading from no association between waist circumference and skin AF in individuals with only central obesity to a significant though limited association in centrally obese with other cardiometabolic risk factors and finally to a strong association in established cardiovascular disease.

Strengths of this study are the large number of people and the wide variety in age, also including younger people. Clinical and biochemical variables already known to influence skin AF were taken into account, as well as some additional ones such as alcohol consumption. Our study population consisted of individuals without known cardiovascular disease and diabetes, including individuals with central obesity both with and without other cardiometabolic risk factors, thereby making it an appropriate population to study the accumulation of AGEs before cardiovascular disease or diabetes become manifest.

Due to the cross-sectional design, our data only indicate associations and not causal relations between skin AF and the variables under study. With regard to age, current smoking, creatinine clearance and current alcohol use, there is sufficient evidence or pathophysiological understanding to assume that they are responsible for a higher, or in case of alcohol, for a lower skin AF. However, for hs-CRP the direction of the association is less clear. A higher skin AF could also be the reason for higher levels of hs-CRP. Both cross-linking of proteins caused by AGEs and receptor mediated cellular activation enhance inflammation and thereby levels of hs-CRP.[1] Both factors are likely to influence and enhance each other.

Although chronic hyperglycemia is known to enhance accumulation of AGEs, we did not find fasting glucose to be independently associated with skin AF. This might be due to the normal mean glucose and the limited variance in this population. Measurement of HbA1c, as a measure of the duration and magnitude of chronic sustained hyperglycemia, might have shown a stronger association with skin AF in our population. However, we did not perform this measurement.

Since the AGE reader is not yet validated in populations with a darker skin type we restricted our analysis to Caucasians with a skin reflectance of at least 6%. Our results are therefore not generalizable to non-Western individuals.

Limitations of the AGE Reader as a measure of tissue AGE accumulation are described in detail elsewhere: not all AGEs exhibit fluorescent properties and will therefore not be measured with the AGE Reader. Other interstitial, cellular and vascular components can exhibit fluorescence as well.[6]

We found a discrepancy in the explained variance between the centrally obese and non-obese group: 29.4% vs. 39.1%. Other factors that were not taken into account in our study might play a role. Food-derived AGEs could be such a factor. AGEs are readily derived from heat-treated foods, especially animal products that have been broiled, grilled or fried. Obese persons often eat more of these products compared with non-obese persons. Uribarri et al. reported that the consumption of dietary AGEs, but not of calories, correlated independently with circulating AGEs.[23] However, serum AGE may not adequately reflect tissue AGE,[24] so the influence of food-derived AGEs on tissue AGE content still remains to be clarified.

In conclusion, although waist circumference is a well-established risk factor for cardiovascular disease and skin AF is associated with cardiovascular disease, in people with an increased waist circumference the contribution of waist circumference to the skin AF's variability was limited and might also be determined by the duration of the central obesity. The relationship between waist circumference and skin AF is not as obvious as we hypothesized.

Patients and Methods

Study population. A cross-sectional study was performed in five general practices in IJsselstein, a small city in the center of the Netherlands.[25] All patients from these practices between 20 and 70 y old and not known with diabetes, hypertension or dyslipidemia were offered the possibility of screening for metabolic syndrome according to the NCEP ATP III criteria by measuring their waist circumference as a first step. The present study is embedded in this screening study. The study was approved by the local ethical committee.

Examinations. Examinations are described in detail elsewhere.[25] All participants had a physical examination to measure body weight, height, blood pressure and waist circumference. Central obesity was defined as a waist circumference above 88 and 102 cm for respectively women and men, according to the NCEP ATP III criteria.[26]

Venous blood samples were drawn after an overnight fast to determine blood glucose, lipids (triglycerides, total, HDL- and LDL-cholesterol), creatinine and high sensitive C-reactive protein (hs-CRP). Urine was collected to assess the albumin to creatinine ratio. Creatinine clearance was calculated by using the Cockroft Gault formula.

All laboratory assays were performed by the local laboratory using standard laboratory methods. A questionnaire was used to determine ethnicity, lifestyle factors (smoking habits, alcohol consumption, and physical activity), relevant medical history, family history type 2 diabetes and socio-economic and demographic variables. Smoking was regarded positive when the participant was currently smoking tobacco; in case of former or never smoking it was regarded negative. Alcohol consumption was classified as 'none' (reference category), "moderate" (1–21 units per week in men or 1–14 units per week in women) and "excessive" (> 21 units per week in men and > 14 units per week in women). Physical activity was assessed using the validated SQUASH questionnaire.[27] Physical activity was regarded positive when a person reported at least 150 min of moderate physical activity per week. Family history of diabetes was regarded as positive if at least one parent or sibling was known with type 2 diabetes before the age of 60.

Patients were advised to contact their general practice for the results of their screening. In case of detected cardiovascular risk factors they received usual care by their general practitioner.[28]

Skin autofluorescence. Skin AF was measured with an AGE Reader (DiagnOptics BV), which is described in more detail elsewhere.[2] In short, the AGE Reader illuminates a skin surface (shielded from surrounding light) on the volar side of the underarm. This area of approximately 4 cm^2 is illuminated with a wavelength of 300–420 nm. Emission light and reflected excitation light from the skin are measured with a spectrometer. By dividing the average light intensity emitted per nm over the 420–600 nm range by the average light intensity emitted per nm over the 300–420 range the autofluorescence is calculated in arbitrary units (AU).

Skin pigmentation may absorb light and thus influence skin AF. To take this into account, skin reflection measurements across the 300–420 nm range are compared with those of a white teflon block (assuming 100% reflectance). Skin reflection can then be calculated by dividing the mean intensity of light reflected from the skin by the mean intensity reflected from the white teflon block across the 300–420 nm range.

Statistical analyses. Since skin AF is affected by skin color and the AGE Reader is only validated in populations with a white skin type, we restricted our analysis to Western Europeans with a skin reflection of at least 6.0%.

Descriptive statistics were used to determine means and proportions. For exponential variables (hs-CRP) a log transformation was performed. To test for differences in characteristics between the centrally obese and non-obese groups, we used Chi-square tests for categorical variables, independent t-tests for normally distributed continuous variables and Mann-Whitney tests for not normally distributed continuous variables. We also determined levels of skin AF in subgroups of either centrally obese or non-obese individuals without other cardiometabolic risk factors. These individuals were selected based on the absence of hypertension, dyslipidemia and impaired glucose (levels of blood pressure, triglycerides, HDL-cholesterol and fasting glucose below the NCEP ATP III criteria thresholds[26] and a total cholesterol < 5.0 mmol/L).

Since age and smoking are the most important factors in determining skin AF, we also evaluated the difference between the centrally obese and non-obese individuals with adjustment for these factors. Mean levels of skin AF in smokers and non-smokers were calculated in both groups with and without central obesity as well as over ten years age categories.

To assess the associations with skin AF, we performed univariable linear regression analyses for all variables mentioned in **Table 1**. In order to get reliable estimates of the regression coefficients, multicollinearity was checked (Pearson correlation coefficient ≥ 0.80) and the variables showing the weakest association with skin AF were excluded from the multivariable analysis. Consequently, diastolic blood pressure and BMI were excluded in favor of systolic blood pressure and waist circumference. All other variables were included.

For multivariable analysis, we created a new variable for waist circumference, taking into account the gender specific thresholds for central obesity. We did this by extracting the gender specific threshold (88 cm for women, 102 cm for men) from the waist circumference value obtained by physical examination. The new variable indicated the number of centimeters the waist circumference measured above or below the threshold for central obesity.

Variables that did not retain significance in the multivariable analysis were subsequently excluded from the model (backward selection). A p value < 0.05 was considered significant. Multivariable analysis was also performed in the subgroup of centrally obese individuals without other cardiovascular risk factors. Analyses were performed using SPSS for Windows version 15.0.

Disclosure of Potential Conflicts of interest

This study was supported by an unrestricted research grant from the Investigator Initiated Studies Program of MSD.

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