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RESEARCH ARTICLE

Advanced glycation end-products (AGEs) and associations with cardio-metabolic, lifestyle, and dietary factors in a general population: the NQplus study

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Abstract

Background: Advanced glycation end-products are a heterogeneous group of molecules that are formed during reactions between reducing sugars and proteins. Advanced glycation end-products are thought to play a role in several diseases, including diabetes mellitus and can be measured non-invasively using skin autofluorescence (AF). The aim of this cross-sectional study was to investigate associations between skin AF and cardio-metabolic, lifestyle, and dietary factors within a general population.

Methods: The NQplus study is an ongoing longitudinal study in the surroundings of Wageningen, the Netherlands. In this cross-sectional study, skin AF was measured in 957 participants, aged 20 to 77 years, with the AGE Reader. Logistic regression was used to assess associations between skin AF and the following factors: demographics, adiposity, blood lipids, fasting glucose, HbA_{1c}, blood pressure, dietary intake, and disease history. Stepwise linear regression was used to identify factors correlating with skin AF.

Results: In males, skin AF was significantly associated with age, coffee intake, systolic and diastolic blood pressure, body fat, full fat dairy, and educational level. In females, significant associations were found with age, coffee intake, HbA_{1c}, diabetes, and eGFR. In the total population, current smoking was positively associated with skin AF.

Conclusions: This cross-sectional study in a general population showed that age and coffee intake were associated with skin AF in both males and females. We observed a gender disparity for some of the associations with skin AF, which need to be confirmed by further research. More detailed research is needed to assess the association between skin AF and diet.

KEYWORDS

advanced glycation end-products, AGEs, skin autofluorescence, nutrition, lifestyle, general population

1 | INTRODUCTION

Advanced glycation end-products (AGEs) are a heterogeneous group of molecules which are formed in the body when the carbonyl group of reducing sugars reacts with free amino-groups on proteins.[1] This non-enzymatic glycation process is known as the Maillard reaction, which was first described by the French chemist Louis-Camille Maillard[2] in 1912. The production of AGEs is part of normal metabolism. During lifetime, AGEs accumulate in the body, including the skin.[3] Normally, AGE formation is a slow and regulated process in vivo.[4] In some conditions, AGE formation is accelerated, for instance during hyperglycaemia and increased oxidative stress.[5,6] In the past decades, researchers began to recognize that AGEs may negatively affect health. Advanced glycation end-products are positively associated with diabetes and its complications[4,7-9] and possibly also with neurodegenerative diseases, such as Alzheimer disease.[10]

Besides endogenous formation of AGEs, it is also suggested that diet influences the amount of AGEs in the body. It has been estimated

that 10% of an AGE-rich meal is absorbed into the serum.[11] These so-called dietary AGEs are mostly present in animal-derived foods, high in fat and protein.[12] Meats contain the highest AGE levels per standard serving size, whereas vegetables, fruits, and whole grains are low in AGEs.[12] The level of AGEs in foods is also influenced by the method of preparation. High temperatures and low humidity accelerate AGE formation. This is the case during grilling, frying, roasting, and baking.[12] Besides foods, cigarette smoking is a source of exogenous AGEs.[13]

Skin autofluorescence (AF) is a relatively new method to measure AGEs. Its use as a tool in clinical practice to estimate health risk, especially in cardiovascular disease (CVD), kidney failure, and diabetes, is subject of research. AGEs in the skin, measured using skin AF, are associated with diabetes and its cardiovascular,[14,15] nephrological,[16] and neurological[17] complications. Moreover, atherosclerosis was also associated with skin AF, independent of diabetes.[18]

The aim of this study is to investigate associations between skin AF and a broad range of cardio-metabolic and lifestyle factors, including age, diabetes, food intake, and cardiovascular mediators within a general, mostly healthy, population. Up till now, most research has focused on associations of AGEs in patient populations, eg, in diabetic participants or participants with CVD, while only a few studies have focused on associations in a general population.[3,19-21]

2 | METHODS

2.1 | Study design

The Nutrition Questionnaires plus (NQplus) study is a longitudinal observational study focused on diet, lifestyle, and health.[22] The study has the objectives to build a national dietary assessment reference database to validate and generate food frequency questionnaires (FFQs) and to build a (bio)database for studies investigating diet, eating behaviour, and health outcomes. Questionnaires, physical examinations, and laboratory assays were performed at baseline, after year 1 and year 2. The current investigation is a cross-sectional study using the baseline measurements.

2.2 | Participants

Between March 2011 and February 2013, a total of 2048 participants, aged 20 to 77, were recruited via random sampling via municipal health centres in the different regions. Focus was on the cities of Ede, Wageningen, Renkum, Arnhem, and Veenendaal within the Netherlands. Skin AF was measured in 1723 mostly healthy Caucasian participants (843 men and 880 women). After excluding persons with missing data on education (n = 8), diabetes (n = 74), CVD (n = 16), smoking status (n = 278), food intake (n = 144), BMI (n = 1), body fat (137), waist circumference (n = 1), eGFR (n = 18), fasting plasma glucose (n = 1), hemoglobin A_{1c} (HbA_{1c}, n = 6), low-density lipoprotein (LDL, n = 2), and physical activity (n = 80), the analytical sample included 957 participants. Analyses investigating food intake included 923 persons.

2.3 | AGE measurements

The AGE accumulation was measured by skin AF using the AGE Reader (Diagnoptics, Groningen, The Netherlands), a non-invasive method to measure AGEs. The AGE Reader uses an ultra-violet-A black light tube, which illuminates approximately 4 cm^2 of the skin. Skin AF was expressed in arbitrary units (AU), which is calculated as the ratio between the average emission intensity per nm in the 420 to 600 nm range and the average excitation intensity per nm in the 300 to 420 nm range, multiplied by 100.[15] Skin AF was measured 3 times at the volar side of the arm and then averaged. During measurements, impurities of the skin like scars and birthmarks were avoided as much as possible, and it was made sure that participants did not have sunscreen on their skin since this could affect the results.[23] Five implausible and 2 extremely high skin AF values were excluded. As a result, for 5 participants, the mean skin AF consisted of 2 measurements and for 1 participant of only 1 measurement.

2.4 | Assessment of cardio-metabolic and lifestyle factors

2.4.1 | Anthropometry

Weight, height, waist circumference, and total body fat were measured during the physical examinations. Weight and height were determined without shoes and heavy upper clothing with a 0.1 decimal accuracy. Waist circumference was measured 2 times in between the lowest rib and the iliac crest, and then averaged. Total body fat was measured using dual-energy X-ray absorptiometry (DXA)(Lunar Prodigy, GE Healthcare).

2.4.2 | Blood parameters

Fasting samples were taken to determine total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, creatinine, HbA_{1c}, and plasma glucose. Using serum creatinine levels, age, and gender, the estimated glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula.[24]

2.4.3 | Blood pressure

A sphygmomanometer was used to measure blood pressure (Omron-HEM 907, Omron Healthcare). The average of 5 measurements after a 5-minute rest was used for analysis.

2.4.4 | Dietary intake

Food intake was assessed with a general, semi-quantitative 180-item FFQ. The FFQ was previously validated for the intake of energy, macronutrients and micronutrients.[25-27] Household measures were used to estimate portion sizes in grams. The intake of the following foods indicated to be rich in AGEs were used in the analysis: meats, full-fat dairy, fats and oils, nuts and seeds, cookies/cakes/pastry, and snacks/fries. Coffee and bread intake were also investigated because they undergo roasting and baking during processing. Recently, caffeine intake was associated with skin intrinsic fluorescence (SIF).[28] For this reason, intake of black and green tea was also investigated.

Furthermore, intake of energy (kcal) and alcohol (g) were included in the analysis to assess if these were related to skin AF.

2.4.5 | Demographics and other lifestyle factors

Self-administered questionnaires provided information about demographics (age, gender, education level, smoking status, and menopausal status), self-reported disease history (diabetes mellitus, kidney disease, CVD), and physical activity using the SQUASH questionnaire.[29]

2.5 | Statistical analysis

Statistical analyses were performed using the statistical software program IBM SPSS Statistics (version 22). An independent sample *t* test was used to test differences between males and females, diabetic and non-diabetic participants and participants with CVD or without CVD.

Logistic regression was used to estimate crude and adjusted odds ratios (ORs) and their 95% CI of having a skin AF above the median for the risk factors of interest. Analyses were performed on the total sample and for men and women separately. Odds ratios were adjusted for age, diabetes (yes, no), smoking status (never, former, current), education level (low/medium, high), and CVD (yes, no). Menopausal status (yes, no) was defined using the complex definition by Phipps et al.[30] Reference values for systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and waist circumference were derived from the European guidelines on cardiovascular risk prevention.[31] Reference values for HbA_{1c} (<5.7%, 39mmol/mol) and fasting plasma glucose (<5.6mmol/l) were derived from the American Diabetes Association.[32] For body fat percentage, cut-off values of ≥25% for males and ≥35% for females were used.[33] Kidney failure was not included in analysis, because the number of participants diagnosed was too low to draw valid conclusions (n = 5), instead eGFR was used as a measure of renal status. An eGFR, <60 mL/min/1.73 m^2, was used as a threshold for a decreased filtration rate of the kidneys.[34] For logistic regression analysis on the intake of food groups of interest, ORs were determined per 50 g of intake, except for alcohol, energy, and oils/fats. Odds ratios for alcohol intake were calculated per 10 g. To calculate the OR for energy intake, a low intake (<2000 kcal for females and <2500 kcal for males) vs high intake (≥2000 kcal for females and ≥2500 kcal for males) was tested. For the intake of oils and fat, ORs were determined per 20 g. For food groups, ORs were adjusted for age, smoking, and energy intake by the residual method.[35]

At last, stepwise linear regression was performed to determine factors correlating with skin AF. The following variables were added to the model: age, gender, smoking status "current," smoking status "former," history of CVD, prevalent diabetes mellitus, body mass index (BMI. kg/m^2), body fat (%), waist circumference (cm), systolic and diastolic blood pressure (mm Hg), total cholesterol (mmol/L), LDL (mmol/L), triglycerides (mmol/L), fasting glucose (mmol/L), HbA_{1c} (%), eGFR (mL/min/1.73 m^2), physical activity (min/wk), sedentary time (min/wk), and the intake (g/d) of energy, alcohol; coffee; black tea; green tea; bread; cookies, pastry, and cake; nuts and seeds; snacks and fries; meats; (full fat) dairy; and intake of fats and oils. In females, menopausal status was additionally included in the model.

3 | RESULTS

Mean skin AF in the total population was 2.02 ± 0.38 AU. Skin AF values ranged from 1.08-3.88 AU. Fifty-one per cent of the participants were male. Mean skin AF was 2.13 ± 0.38 AU in men and 1.90 ± 0.38 AU in women (Table 1). On average, men were

TABLE 1 Baseline characteristics of 957 participants from the Nutrition Questionnaires plus study overall and across tertiles of skin autofluorescence (AF)

	Males	Tertiles of skin AF			Females	Tertiles of skin AF		
Characteristic	Overall	First	Second	Third	Overall	First	Second	Third
N	488	108	167	213	469	211	152	106
Skin AF, AU	2.13 ± 0.38	1.66 ± 0.15	2.01 ± 0.09	2.47 ± 0.27	1.90 ± 0.35	1.60 ± 0.18	2.00 ± 0.09	2.37 ± 0.20
Min-max	1.10-3.88	1.10-1.84	1.84-2.17	2.17-3.88	1.08-3.13	1.08-1.84	1.84-2.16	2.17-3.13
Age, y	55.8 ± 10.2	46.5 ± 11.8	55.7 ± 8.3	60.6 ± 6.9	51.4 ± 11.2	45.7 ± 11.7	55.0 ± 8.5	57.5 ± 7.7
Education level, n (%)								
High	309 (63)	78 (72)	113 (68)	118 (55)	294 (63)	145 (69)	86 (56)	64 (60)
Intermediate	145 (30)	25 (23)	45 (27)	75 (35)	154 (33)	60 (28)	60 (39)	34 (32)
Low	34 (7)	5 (5)	9 (5)	20 (9)	21 (4)	6 (3)	7 (5)	8 (8)
Diabetes mellitus, n (%)	20 (4)	2 (2)	6 (4)	12 (6)	14 (4)	0 (0)	5 (3)	9 (8)
CVD, n (%)	19 (4)	1 (1)	2 (1)	16 (8)	8 (2)	1 (0)	3 (2)	4 (4)
Smoking status, n (%)								
Never	225 (46)	70 (65)	81 (49)	74 (35)	282 (60)	150 (71)	76 (50)	56 (53)
Former	219 (45)	29 (27)	78 (47)	112 (53)	160 (34)	51 (24)	66 (43)	43 (41)
Current	44 (9)	9 (8)	8 (5)	27 (13)	27 (6)	10 (5)	10 (7)	7 (7)

Abbreviations: AU, arbitrary units; CVD, cardiovascular disease (heart attack or stroke).

Shown as n (%), mean ± SD or percentage.

55.8 ± 10.1 years old, and women were 51.4 ± 11.2 years old. Furthermore, the population was generally healthy: of the 957 participants, 4% reported to have diabetes mellitus (type 2: n = 30, type 1: n = 2, other or unknown: n = 2) and 3% reported a CVD event (heart attack or stroke). Most participants had a high education level (63%).

Age and skin AF were positively correlated (r = 0.568, P = <.001). Mean skin AF differed significantly between genders: Skin AF in male participants was 0.23 AU higher (P = <.001). Mean skin AF in non-diabetic participants was 2.01 ± 0.38 and 2.30 ± 0.34 in diabetic participants (P = <.001). Mean skin AF in participants without CVD history was 1.98 ± 0.38 and 2.42 ± 0.46 in participants with CVD history (P = <.001).

A 1-year higher age was associated with an 11% higher odds for men and a 9% higher odds for women for having a skin AF above the median, after adjustment for smoking status, diabetes, education level, and CVD: OR (95% CI) 1.11 (1.08, 1.14) in men and 1.09 (1.07, 1.12) in women (Table 2). Diabetic female participants had a 6.14 higher odds of having a high skin AF compared to non-diabetic participants when adjusted for age, smoking status, education level,

TABLE 2 Crude and adjusted odds ratios (ORs) and 95% confidence interval (CI) of having a high skin AF and age, education, and health outcomes in 957 adults from the Nutrition Questionnaires plus study

		Males (n = 488)		Females (n = 469)	
Variable		Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Age[a]	Per year	1.12 (1.09, 1.15)	1.11 (1.08, 1.14)	1.10 (1.07, 1.12)	1.09 (1.07, 1.12)
Menopausal status[a]	Yes No	-[c]	-[c]	5.14 (3.34, 7.90) 1.00 (ref.)	1.37 (0.71, 2.62) 1.00 (ref.)
Education level[a]	Low and medium High	1.00 (ref.) 0.57 (0.38, 0.85)	1.00 (ref.) 0.64 (0.40, 1.00)	1.00 (ref.) 0.69 (0.47, 1.00)	1.00 (ref.) 0.85 (0.56, 1.29)
Diabetes mellitus[a]	Yes No	3.20 (0.93, 11.09) 1.00 (ref.)	1.81 (0.48, 6.91) 1.00 (ref.)	8.68 (1.92, 39.22) 1.00 (ref.)	6.14 (1.31, 28.71) 1.00 (ref.)
CVD[a]	Yes No	10.33 (1.37, 78.05) 1.00 (ref.)	4.51 (0.57, 35.84) 1.00 (ref.)	2.31 (0.55, 9.80) 1.00 (ref.)	0.79 (0.17, 3.66) 1.00 (ref.)
Smoking[a]	Current Former Never	2.58 (1.24, 5.36) 2.44 (1.64, 3.64) 1.00 (ref.)	1.86 (0.82, 4.22) 1.26 (0.79, 2.00) 1.00 (ref.)	1.66 (0.75, 3.68) 1.98 (1.34, 2.94) 1.00 (ref.)	1.77 (0.73, 4.29) 1.34 (0.86, 2.08) 1.00 (ref.)
Body mass index, kg/m^2[a]	<25 25-30 ≥30	1.00 (ref.) 1.52 (1.02, 2.26) 2.54 (1.31, 4.92)	1.00 (ref.) 1.09 (0.69, 1.74) 1.45 (0.69, 3.05)	1.00 (ref.) 1.42 (0.94, 2.16) 1.95 (1.14, 3.34)	1.00 (ref.) 1.06 (0.67, 1.69) 1.48 (0.81, 2.69)
Fat mass (%)[a]	<♂: 25, ♀: 35 ≥♂: 25, ♀: 35	1.00 (ref.) 1.43 (0.99, 2.08)	1.00 (ref.) 1.14 (0.74, 1.75)	1.00 (ref.) 1.75 (1.21, 2.54)	1.00 (ref.) 1.49 (0.99, 2.26)
Waist circumference (cm)[a]	<♂: 102, ♀: 88 ≥♂: 102, ♀: 88	1.00 (ref.) 2.27 (1.44, 3.59)	1.00 (ref.) 1.26 (0.73, 2.18)	1.00 (ref.) 1.42 (0.79, 2.56)	1.00 (ref.) 0.93 (0.47, 1.81)
eGFR, mL/min/1.73 m^2[a]	≥60 <60	1.00 (ref.) 3.07 (0.67, 13.99)	1.00 (ref.) 1.16 (0.24, 5.66)	-[d]	-[d]
	Per unit	0.95 (0.94, 0.97)	1.00 (0.98, 1.02)	0.95 (0.94, 0.97)	0.98 (0.97, 1.00)
HbA_{1c}, mmol/mol[b]	<5.7% (39mmol/mol) ≥5.7% (39mmol/mol)	1.00 (ref.) 2.01 (1.20, 3.38)	1.00 (ref.) 1.06 (0.59, 1.90)	1.00 (ref.) 3.37 (2.08, 5.45)	1.00 (ref.) 1.83 (1.08, 3.09)
Fasting glucose, mmol/L[b]	<5.6 ≥5.6	1.00 (ref.) 1.58 (1.08, 2.29)	1.00 (ref.) 1.10 (0.72, 1.70)	1.00 (ref.) 1.89 (1.28, 2.78)	1.00 (ref.) 1.24 (0.81, 1.91)
Systolic BP, mm Hg[a]	<140 ≥140	1.00 (ref.) 1.04 (0.68, 1.58)	1.00 (ref.) 0.52 (0.31, 0.86)	1.00 (ref.) 1.31 (0.73, 2.33)	1.00 (ref.) 0.55 (0.28, 1.06)
Diastolic BP, mm Hg[a]	<90 ≥90	1.00 (ref.) 0.85 (0.45, 1.61)	1.00 (ref.) 0.73 (0.37, 1.46)	1.00 (ref.) 0.77 (0.32, 1.88)	1.00 (ref.) 0.57 (0.22, 1.51)
Total cholesterol, mmol/L[a]	<5.0 ≥5.0	1.00 (ref.) 1.26 (0.86, 1.85)	1.00 (ref.) 1.04 (0.65, 1.66)	1.00 (ref.) 2.10 (1.40, 3.15)	1.00 (ref.) 1.04 (0.63, 1.72)
HDL, mmol/L[a]	<♂: 1.0, ♀: 1.2 ≥♂: 1.0, ♀: 1.2	1.00 (ref.) 0.96 (0.55, 1.69)	1.00 (ref.) 0.67 (0.34, 1.29)	1.00 (ref.) 1.95 (0.88, 4.31)	1.00 (ref.) 1.83 (0.70, 4.77)
LDL, mmol/L[a]	< 3.0 ≥ 3.0	1.00 (ref.) 1.10 (0.75, 1.62)	1.00 (ref.) 1.14 (0.71, 1.82)	1.00 (ref.) 1.61 (1.11, 2.35)	1.00 (ref.) 0.88 (0.56, 1.39)
Triglycerides, mmol/L[a]	<1.7 ≥1.7	1.00 (ref.) 1.29 (0.81, 2.04)	1.00 (ref.) 1.07 (0.63, 1.83)	1.00 (ref.) 1.28 (0.74, 2.20)	1.00 (ref.) 0.89 (0.48, 1.64)
Total PA, min/wk[a]	Per 100 units	0.97 (0.96, 0.99)	1.00 (0.98, 1.02)	0.98 (0.96, 1.00)	1.01 (0.99, 1.03)
Sedentary time, min/wk[a]	Per 100 units	1.00 (0.99, 1.02)	0.99 (0.98, 1.01)	1.00 (0.99, 1.01)	1.00 (0.98, 1.01)

Abbreviations: BP, blood pressure; CVD, cardiovascular disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PA, physical activity.

[a]ORs adjusted for age, diabetes mellitus, smoking status, education level and CVD.

[b]ORs adjusted for age, smoking status, education level and CVD.

[c]Not applicable to men.

[d]The OR could not be determined because the subgroup size was too small.

and CVD (95% CI, 1.31, 28.71). In the total population, current smoking was associated with a 1.84 times higher odds of being in the high skin AF category compared to non-smokers (95% CI, 1.01, 3.34). In the analysis per gender, the association between current smoking and skin AF was not significant anymore: OR males: 1.86, 95% CI, 0.79, 4.22), OR females: 1.77, 95% CI, 0.73, 4.29). Hemoglobin $A1_c$ was associated with skin AF in women only (adjusted OR: 1.83, 95% CI: 1.08; 3.09). A systolic blood pressure ≥140 mm Hg was related to a 48% lower odds in men (OR: 0.53, 95% CI: 0.31; 0.86) after adjustment for age, diabetes, smoking status, education level, and CVD. The association of skin AF with systolic blood pressure was inverted after adjustment. In women, this association was not significant (adjusted OR: 0.55, 95% CI: 0.28; 1.06).

An increased intake of 50 mL of coffee per day increased the odds of having high skin AF by 7% in men and 6% in women after adjustment for age, smoking status, education level, and CVD: OR 1.07 (95% CI, 1.03, 1.11) in men and OR 1.06 (95% CI, 1.02, 1.10) in women (Table 3). For the intake of meat, which contains relatively high levels of AGEs, the adjusted OR was 1.15 (95% CI, 0.87, 1.52) in men and 1.18 (95% CI, 0.88, 1.59) in women. However, this association was not significant. For other food groups of interest, also no significant association with high skin AF was observed.

Stepwise linear regression revealed that in men, skin AF correlated with age, CVD, coffee intake, body fat, diastolic blood pressure, full fat dairy, and education level (Table 4). In women, skin AF correlated with, age coffee intake, diabetes, and eGFR. Together, these factors explained 37% of the inter-individual variance in men and 35% in women. Age showed the strongest correlation with skin AF, explaining approximately 29% of the variation in men and 30% in women.

4 | DISCUSSION

In this cross-sectional study among 957 adult participants from the general Dutch population, associations between a broad range of demographic, cardio-metabolic, dietary, and lifestyle risk factors were analysed in relation to skin AF, a measure of AGEs accumulation. In men, skin AF was associated with age, intake of coffee, systolic and diastolic blood pressure, body fat, full fat dairy and educational level. For women, significant associations were found with age, intake of coffee, HbA_{1c}, diabetes, and eGFR. In the total population, current smoking was also positively associated with skin AF.

Recently, a cross-sectional study by van Waateringe et al among 8695 Dutch individuals without diabetes from the general population showed that skin AF was associated with age, BMI, HbA_{1c}, creatinine clearance, smoking, and coffee consumption; which is, with the exception of BMI, in line with our findings.[21] Although the study from van Waateringe et al had a larger sample size, the current study used an FFQ to assess dietary intake and DXA to measure fat mass.

The observed relationship of skin AF with age was expected; this was documented before in healthy participants by several other studies.[3,19-21,36,37] Cigarette smoking, an exogenous source of AGEs, has been previously associated with skin AF.[3,19-21,37] In the total population, a significant association between skin AF and smoking status "current" was observed; however, in sex-specific analysis, this association was not significant anymore.

Other studies in healthy participants did not detect strong gender differences. When other studies found a difference, then females tended to have higher skin AF; however, in the current study, females on average had lower skin AF. It is possible that the observed difference in skin AF between genders in the current study was influenced by other unknown factors. Based on the results of this study, it cannot be concluded whether the observed gender difference is a true difference or due to chance. Koetsier and colleagues conducted a cross-sectional study in 428 healthy Dutch participants. Here, a higher skin AF was found in smoking females only.[3] Another cross-sectional study by Yue et al in 1265 healthy Chinese participants did not find gender differences in skin AF levels.[20] Klenovics et al conducted a cross-sectional study in 1385 Slovak participants; a higher skin AF in women was observed only in the age category of 40 to 49 years.[19] In a cross-

TABLE 3 Association of having a skin autofluorescence above the median with the intake of energy, alcohol and food groups of interest in 923 adults without diabetes from the Nutrition Questionnaires plus study

		Males (n = 488)		Females (n = 469)	
Intake of (per d)		Crude OR (95% CI)	Adjusted OR (95% CI)[a]	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Energy, kcal	≥♂: 2500, ♀: 2000	0.57 (0.38, 0.85)	0.86 (0.54, 1.37)	0.95 (0.65, 1.40)	1.08 (0.70, 1.65)
Alcohol, g	Per 10 g	1.04 (0.90, 1.21)	0.84 (0.70, 1.00)	1.19 (0.98, 1.44)	0.94 (0.76, 1.17)
Coffee, mL	Per 50 g	1.07 (1.03, 1.10)	1.07 (1.03, 1.11)	1.08 (1.04, 1.12)	1.06 (1.02, 1.10)
Black tea, mL	Per 50 g	0.99 (0.93, 1.06)	0.95 (0.88, 1.02)	0.96 (0.90, 1.02)	0.95 (0.88, 1.02)
Green tea, mL	Per 50 g	0.92 (0.85, 1.01)	0.96 (0.86, 1.06)	0.97 (0.91, 1.03)	1.02 (0.95, 1.10)
Bread, g	Per 50 g	1.07 (0.91, 1.26)	1.13 (0.94, 1.36)	1.00 (0.82, 1.23)	0.99 (0.79, 1.24)
Pastry, cakes, and cookies, g	Per 50 g	1.09 (0.76, 1.57)	1.21 (0.79, 1.84)	0.70 (0.44, 1.12)	0.80 (0.46, 1.38)
Full fat dairy, g	Per 50 g	1.06 (0.93, 1.20)	1.05 (0.91, 1.20)	1.01 (0.90, 1.14)	0.99 (0.87, 1.13)
Oils, fats, and dressings, g	Per 20 g	0.80 (0.62, 1.03)	0.77 (0.57, 1.04)	1.08 (0.82, 1.42)	1.08 (0.79, 1.47)
Nuts and seeds, g	Per 50 g	1.12 (0.68, 1.86)	0.95 (0.54, 1.69)	1.18 (0.68, 2.03)	1.06 (0.58, 1.93)
Snacks and fries, g	Per 50 g	0.60 (0.46, 0.79)	0.94 (0.70, 1.25)	0.65 (0.45, 0.95)	1.06 (0.69, 1.63)
Meats, g	Per 50 g	1.17 (0.92, 1.49)	1.15 (0.87, 1.52)	1.06 (0.81, 1.38)	1.18 (0.88, 1.59)

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]ORs adjusted for energy, age, smoking status, education level, and CVD.

TABLE 4 Summary of stepwise linear regression analysis of skin autofluorescence (n = 957)

Model	Unstandardized Coefficients		Standardized Coefficients			
	B	SE	β	*P* value	R^2	Cumulative R^2
Males (n = 488)						
Constant	1.239	0.137		<.001		
Age, y	0.018	0.001	.490	<.001	0.295	0.295
CVD (yes/no)	0.335	0.072	.170	<.001	0.060	0.355
Coffee intake (per 50 mL/d)	0.009	0.002	.155	<.001	0.028	0.383
Body fat, %	0.008	0.002	.133	.001	0.041	0.424
Diastolic BP, mm Hg	−0.005	0.001	−.118	.002	0.001	0.425
Full fat dairy (per 50 g/d)	0.023	0.009	.093	.011	0.011	0.436
Education (low/medium vs high)	−0.063	0.029	−.079	.029	0.014	0.450
Females (n = 469)						
Constant	1.291	0.156		<.001		
Age, y	0.014	0.001	.457	<.001	0.308	0.308
Coffee intake (per 50 mL/d)	0.010	0.002	.160	<.001	0.070	0.378
Diabetes mellitus	0.280	0.076	.139	<.001	0.034	0.412
eGFR, mL/min/1.73 m^2	−0.002	0.001	−.091	.049	0.147	0.558

Abbreviations: BP, blood pressure; CVD, cardiovascular disease (heart attack or stroke); eGFR, estimated glomerular filtration rate.

sectional study in 973 diabetic participants and 231 controls, Lutgers et al observed that female gender was positively associated with skin AF in diabetic participants only.[37]

Diabetic participants had higher levels of skin AF compared to non-diabetic participants. This is what one would expect; hyperglycaemia and oxidative stress are believed to enhance endogenous AGE formation.[6] In analyses stratified by gender, the association of skin AF and diabetes was significant in women only. Previously, it has been shown that skin AF is significantly higher in diabetic participants than in non-diabetic participants; diabetes duration correlated with skin AF.[37] Levels of HbA_{1c} ≥ 5.7% (39mmol/mol) were associated with skin AF in female participants only. Previously, HbA_{1c} was found to correlate with skin AF in diabetic participants only.[37] Meerwaldt et al also observed a relationship between HbA_{1c} and skin AF in diabetic participants.[36]

The eGFR correlated negatively with skin AF in women. However, the group with a low eGFR in this population was small. Only 20 participants had an eGFR < 60 mL/min/1.73 m^2. Because the kidneys are important for AGE excretion, an association of skin AF with eGFR is in line with evidence from studies investigating AGEs in the kidney during kidney disease. Advanced glycation end-products are cleared less efficient, and they accumulate in the kidney during kidney failure, altering filtration function.[7] Also, more oxidative stress is present.[7] Previously, it was observed that skin AF was negatively correlated with eGFR in a cross-sectional study with 304 pre-dialysis chronic kidney disease patients.[38] Further investigation of the association of skin AF with nutritional intake of AGE-rich food items in participants with kidney failure in a larger study would be interesting.

Serum cholesterol (total, HDL, LDL) and triglyceride levels, important mediators of CVD risk, were not associated with skin AF. In male participants, CVD correlated positively with skin AF. The group of female participants with CVD was small (n = 8). In men, systolic blood pressure was positively associated with skin AF, a relationship that became inverse after correction for age, diabetes, and smoking, indicating that this association was highly influenced by confounding factors. Diastolic blood pressure correlated negatively too with skin AF in men. Several studies linking CVD to skin AF have been performed. For example, skin AF was higher in subclinical atherosclerosis participants than controls, independent of diabetes status.[18] Moreover, in a prospective cohort study of 5 years in patients with peripheral artery disease, skin AF was linked to an increased incidence of cardiovascular events and mortality.[14] In diabetic participants, HDL cholesterol correlated negatively with skin AF.[37]

A positive association between skin AF and coffee intake was observed. This was also observed by van Waateringe et al, who found a dose-dependent positive association between the number of cups of coffee daily and skin AF levels, both in non-diabetic and type 2 diabetes individuals.[21] Moreover, a cross-sectional study in 1.181 diabetes type 1 patients found a positive association between SIF and caffeine consumption.[28] Skin intrinsic fluorescence was also associated with the consumption frequency of coffee and diet coke.[28] Decaffeinated coffee was not associated with SIF, although levels of intake were low in the study population.[28] Increased levels of fluorescence in the skin can be caused by the fluorophores present in coffee. Roasting of coffee beans during processing could contribute to AGE formation, due to the Maillard reaction, whereas caffeine possesses fluorescent properties as well.[28,39] Furthermore, the N-acetyltransferase 2 (NAT2) gene, involved in caffeine metabolism, has been associated with skin AF.[21] However, which of the fluorophores present in coffee result in higher skin AF remains to be determined by further research. In men, full fat dairy intake correlated with skin AF. Previously, it was indicated that animal-derived foods high in fat and protein are a source of dietary AGEs.[12] In the current study, an adjusted OR of 0.84 was found for alcohol intake in men and 0.94 in women, though these results were not statistically significant. Although evidence is limited,

in a cross-sectional study in 816 participants with central obesity and 431 controls, moderate alcohol intake was associated with lower skin AF in both groups.[40] Furthermore, an animal study suggested that a moderate alcohol intake could inhibit AGE formation.[41] Two 16-week trials investigating the effects of an AGE-restricted diet have shown that diet can affect serum AGE levels. In a randomized intervention study in 30 healthy participants, a significant reduction was observed in serum Nε-(carboxymethyl)lysine (CML), an AGE, and AGE-precursor methylglyoxal (MG) after a low-AGE diet.[42] Participants were instructed to prepare food using methods which result in low levels of new AGE formation but did not receive advice as to which products to choose. Advanced glycation end-product receptor 1 (AGER1) and receptor for AGE (RAGE) levels were also reduced. Besides crosslinking of proteins,[43] binding of AGEs to the RAGE is one of the mechanisms which can result in pathogenic effects.[4,44] It induces activation of the NF-κB controlled genes resulting in oxidative stress, thrombogenesis and vascular inflammation.[7,44] There is discussion about to what extent RAGE interacts with AGEs in vivo.[45] Advanced glycated end-product receptor 1 is believed to inhibit the effects of AGE-RAGE interaction.[4] Also 8-isoprostanes, TNF-α and VCAM-1 levels, which mark oxidative stress/inflammation, were reduced. The other trial, performed in 18 healthy and 18 diabetic participants, found similar results concerning serum CML and MG levels, 8-isoprostanes and TNF-α after an AGE-restricted diet.[46] However, potential benefits of dietary AGE restriction need more investigation before conclusions can be drawn. Most studies were small and of short duration. Therefore, future research should further investigate the relationship between dietary intake and skin AF, including a more precise measurement of AGE-rich food intake as well as preparation methods. An FFQ focused on AGE-rich food items, preparation methods, caffeine/coffee, and alcohol intake could be developed to investigate associations.

Body mass index, waist circumference, fasting glucose, and physical activity were not associated with skin AF. Lutgers et al found BMI to correlate with skin AF in diabetic participants but not in healthy controls.[37] Also, Klenovics et al did not find a relationship with BMI in healthy participants.[19] Body fat correlated positively with skin AF in males. In contrast to BMI, which is frequently used as an indicator of overweight/obesity, the measurement of body fat gives a more accurate estimation for body composition. More studies should confirm a possible association between skin AF and body fat percentage. In contrast to our results, Klenovics and colleagues found an association with frequency of exercise per week.[19] Participants with an exercise frequency of ≥3 had a lower skin AF than those who excised 1 to 2 per week. The current study did not find an association with skin AF and physical activity in minutes per week. In the current study, physical activity was estimated using the SQUASH questionnaire. In large populations, this questionnaire can give an indication of physical activity.[29]

Measuring skin AGEs using skin AF is a non-invasive method. Skin AF measurements were validated with the autofluorescence reader, the precursor of the AGE Reader, in 46 type I and II diabetic- and 46 control participants.[36] Skin AF was validated against skin AGEs in homogenized skin biopsies.[36] Skin biopsy samples were analyzed for specific AGEs pentosidine, Nε-(carboxyethyl)lysine (CEL) and CML.[36] Only fluorescent AGEs, e.g. pentosidine, can be measured using skin AF. In the validation study, significant correlations between the non-fluorescent AGEs and pentosidine were observed.[36] The results from the AGE Reader and autofluorescence reader are regarded to be similar.[3] It is not possible to identify specific AGEs using skin AF. Immunological and biochemical assays can be used to measure both fluorescent and non-fluorescent AGEs in skin biopsies. These techniques are relatively complex. Measurements can also be performed in urine- and blood samples, but this does not have to reflect tissue AGEs.[36]

Strengths of the study include the measurement of a broad range of demographic, health, and lifestyle factors in a large population. Moreover, it was possible to correct for several confounders. This is one of the first studies investigating the association of dietary intake and AGE accumulation using skin AF. Also, the association of skin AF and body composition measured using DXA has not been investigated before. Furthermore, this is also one of the few studies investigating a general population, making the results more applicable to the general public than previous studies that have mainly focused on patient populations.

This study also has some limitations. The FFQ was not specifically designed to assess intake of AGEs. Urribari et al indicated that the method of food preparation is an important factor for AGE formation.[12] This information was not part of the FFQ. Next, due to the cross-sectional nature of the study, no causal relationships could be determined. Finally, the results from this study can only be generalized to a general Caucasian population. Due to strong pigmentation of the skin, reflectance levels are too low for a valid measurement in persons with skin type Fitzpatrick type VI (sub-Saharan African- and Afro-American skin tone).[14]

In conclusion, among a wide range of demographic, cardiometabolic, dietary and lifestyle factors age and coffee intake were positively associated with skin AF in both men and women in this general population. Age correlated strongest with skin AF. Positive associations of skin AF with HbA_{1c}, diabetes, eGFR, systolic and diastolic blood pressure, body fat, full fat dairy, and educational level differed in their presence between men and women. These factors should be taken into account in further research on skin AF or when using skin AF as a screening tool in high-risk populations.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

DS and EF designed the study; JV, AG, and EF acquired the data; NB conducted the analysis, interpreted the data, and drafted the article. All authors interpreted the data, critically reviewed the article for important intellectual content and gave final approval of the version to be published.

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