Relation between food and drinking habits, and skin autofluorescence and intima media thickness in subjects at high cardiovascular risk

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Summary

We investigated the relations between food and drinking habits, and estimated exogenous advanced glycation end products (AGE) intake, skin autofluorescence (AF) as a marker of AGE accumulation, and intima media thickness (IMT). IMT of the carotid artery and skin AF were measured in 147 elderly subjects at an increased cardiovascular risk. Food and drinking habits and cardiovascular risk factors were recorded. Intake of exogenously derived AGE was estimated from food diaries. Wine consumption was inversely related with skin AF and IMT of the common carotid artery. The intake of proteins was independently or negatively related to skin AF. Higher skin AF was found in the group that used predominantly margarine or butter. A positive relation was found between unsaturated fat intake and the mean IMT of the carotid bulb. The calculated intake of exogenously diet-derived AGE did not relate with skin AF. Skin AF did not relate to IMT. In conclusion, consumption of wine, and a diet consisting of a high amount of proteins and a low amount of saturated fat, was associated with lower skin AF. Intake of exogenously diet-derived AGE did not relate with skin AF.

Keywords

skin autofluorescence; intima media thickness; food habits; drinking habits; advanced glycation end products; cardiovascular risk factors

The reaction of reducing saccharides with proteins or amino acids produces Maillard reaction products. These are formed during the preparation of food, e.g. during heating, but are also formed in the human body, leading to advanced glycation end-products (AGE) [1]. AGE are the irreversible products of non-enzymatic glycation and oxidation of proteins and lipids. AGE are accumulated as a result of endogenous formation, by exogenous supply and by reduced excretion during renal failure.

Endogenous sources of AGE are oxidative and glycemic stress, and hyperlipidemia [2–4]. Exogenous sources of AGE are smoking [5] and food [6, 7]. Depending mainly on the method of food processing, fats in food contain high amounts of AGE, while foods containing mainly saccharides usually contain lower amounts of AGE. High AGE values are also observed in fried meat. Questionnaires have been developed by Uribarri and Goldberg et al. to provide an estimation of the AGE intake taking into account both their content and the way of preparation of food [8].

AGE accumulate gradually in long-lived tissues with aging [9, 10]. The rate of AGE accumulation is influenced by genetic factors [11, 12]. AGE are implicated in the pathogenesis of atherosclerosis [13], Alzheimer's disease [14], chronic complications of diabetes mellitus [15, 16] and renal failure [17]. High AGE levels derived from AGE consumption may induce inflammation [18] probably through binding of AGE to the AGE receptor (RAGE). RAGE induces a significant activation of the transcription factor, nuclear factor κB (NF-κB) [19–22]. High AGE consumption is associated with an increase in serum C-reactive protein, peripheral

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blood mononuclear cell-derived tumor necrosis factor (TNF-α) and the serum vascular adhesion molecule VCAM-1 [23].

The autofluorescence reader (AFR) is an instrument facilitating estimation of AGE levels in tissues non-invasively. Based on the fluorescence from a part of the various AGE molecules, AFR has been validated in various conditions against levels of collagen-linked fluorescence and several specific AGE in dermal tissue biopsies [24]. Skin autofluorescence (AF), measured by AFR at the arm, is an independent predictor of cardiovascular disease in renal failure [25] and in diabetes mellitus, and an independent predictor of other diabetic complications [26]. Skin AF was also found to be related to markers of oxidative stress in acute coronary disease [27]. Skin AF was moderately associated with intima media thickness (IMT) of the carotid artery in persons with a high risk of cardiovascular disease (CVD) [28], and in autoimmune diseases like rheumatoid arthritis [29], systemic lupus erythematodes and morbus Wegener [30, 31]. IMT is an accepted marker for generalized atherosclerosis burden and CVD risk [32]. Carotid IMT is an independent predictor of transient cerebral ischemia attack, stroke and coronary events, such as myocardial infarction [33–36].

Our aim was to investigate the hypothesis that food patterns and exogenously derived AGE, in particular assessed by a food diary, and smoking history relate with skin AF, using AFR, and with IMT of the carotid artery in subjects at high CVD risk.

MATERIALS AND METHODS

Subjects

From February 2006 until May 2006, we performed our study in men and women from the northern part of the Netherlands, who had previously been recruited as part of the IMPROVE "Carotid Intima Media Thickness (IMT) and IMT-Progression as Predictors of Vascular Events in a High Risk European Population" study.

Inclusion criteria were age between 55 and 79 years and the presence of at least three of the following vascular risk factors:
- male sex or at least 5 years after menopause for women;
- hypercholesterolemia (calculated low-density lipoprotein (LDL)-cholesterol blood levels > 160 mg·dl⁻¹, or treatment with lipid-lowering drugs);
- hypertriglyceridemia (fasting triglyceride levels in blood > 200 mg·dl⁻¹ after diet, or treatment

with triglyceride-lowering drugs);
- hypoalphalipoproteinemia (fasting high-density lipoprotein (HDL)-cholesterol < 40 mg·dl⁻¹);
- hypertension (diastolic blood pressure > 90 mmHg and/or systolic blood pressure > 140 mmHg or treatment with anti-hypertensive drugs);
- diabetes mellitus (fasting blood glucose level > 110 mg·dl⁻¹, or treatment with insulin or oral hypoglycemic drugs);
- smoking habits (at least 10 cigarettes/day for at least thirty months),
- family history of CVD. Family history is defined as first-degree family with CVD (mother or sister before 60 year, father or brother before 50 year).

Exclusion criteria were history of myocardial infarction, angina pectoris, stroke, transient cerebral ischemic attack, or re-vascularization of carotid, coronary or peripheral arteries; aortic aneurysm or claudication; congestive heart failure (III-IV NYHA Class), and history of serious medical conditions that might limit longevity. Additional exclusion criteria concerning the skin AF measurement were a skin phototype V/VI and other skin abnormalities that might have limited the measurement of skin AF.

History of known diabetes mellitus, drug treatment, menopausal status, as well as smoking and food habits were obtained by questionnaires.

Food habit assessment considered quantities and type of alcoholic beverages (wine, beer and spirits), coffee, tea, and milk, as well as consumption of various kinds of fat, meat, fish, eggs and fruits. Fat usage was divided in the categories no usage, or use of mainly seed oil, olive oil, margarine and butter, respectively. A three-day food diary in a randomly selected subgroup of the participants provided the basis for estimation of diet-derived AGE.

The calculation of the diet-derived AGE from this food diary is based on the information of The Netherlands Nutrition Center (Den Haag, The Netherlands) and on tables from GOLDBERG et al. [8], who measured AGE in commonly consumed foods.

Systolic and diastolic blood pressure, body weight and height were measured. Blood samples were taken in the fasting state. Blood glucose, leukocyte counts, serum creatinine, C-reactive protein, uric acid and lipids (total, HDL-cholesterol, triglycerides) were measured by standard laboratory methods. LDL-cholesterol blood levels were calculated by the Friedewald formula [37]. We used the Framingham risk score (FRS) [38] and

the SCORE risk estimation [39] to assess the individual CVD risk. The SCORE risk was assessed by using the chart for populations at low CVD risk.

Approval for the study was obtained from the University Medical Center Groningen Medical Ethics Committee (METC). All subjects gave written informed consent.

Skin autofluorescence (AF) measurements

Skin AF was assessed by AFR (prototype of the current AGE Reader; DiagnOptics, Groningen, The Netherlands). This is a recently validated device, which illuminates approximately 4 cm^2 of skin (protected against the surrounding light) with a light source of 300 nm to 420 nm (peak excitation, ~360 nm) [24–27]. Light from the skin is measured between 300 nm and 600 nm with a spectrometer (AvaSpec 2048 spectrometer; Avantes; Eerbeek, The Netherlands) with a 200 μm UV-VIS glass fiber (Avantes, Eerbeek, The Netherlands). Skin AF was calculated by dividing the average light intensity emitted per nm over the range from 420 nm to 600 nm by the average light intensity emitted per nm over the range from 300 nm to 420 nm. Skin pigmentation influences the AF measurement, in particular if the reflection is below 10%. Therefore, skin AF values were not used in this study when the skin reflection was below 10% [40]. Calculations of AF were performed by the instrument and were observer-independent.

Skin AF was measured at two different volar sides of the arm, approximately 10 cm below the elbow, at both the left and the right arm. Care was taken to measure at normal skin sites. Skin AF is expressed in arbitrary units (AU) and multiplied by 100 for easier evaluation. Repeated skin AF measurements on the same day in control subjects and diabetic patients showed an overall Altman error of 5.03%. Intra-individual seasonal variance among control subjects and diabetic patients showed an Altman error of 5.87% [24].

Intima media thickness (IMT) measurements

The scan protocol included the bilateral recording of the far wall of the common carotid artery in the last part before the bulb (CCA), of the bifurcation or bulb (CB) and of the first proximal centimeter of the internal carotid artery (ICA). The ultrasound parameter used for the statistic analyses was the average of the IMT assessments, measured on the far wall of the left and right common carotid artery (mean IMT CCA) or ICA (mean IMT ICA) or CB (mean IMT CB). Ultrasound scanning of the carotid arteries was performed by expert sonographers, blinded for subject characteristics, using a high-resolution

ESAOTE ultrasound system, model TECHNOS (Genoa, Italy), equipped with a 5–10 Mhz linear array probe.

Statistics

Kolmogorov–Smirnov test was used to assess the normality of the data. Paired data were analysed with the paired Student t-test. Group differences were tested with ANOVA and unpaired Student t-test for normal distribution, and the Kruskal-Wallis test and Mann-Whitney U test for non-normally distributed data. Categorical variables were compared with the χ2-test. For

Tab. 1. Clinical characteristics of the 147 participants.

Clinical characteristic	Value
Age	61 (58 – 68) years
Male	69 persons (47%)
Body mass index	27.7 (25.7 – 31.3) kg.m^{-2}
Waist circumference	(100 ± 14) cm
Current smoking	26 persons (18%)
Previous smoking	68 persons (46%)
Hypercholesterolemia	85 persons (58%)
Hypertriglyceridemia	25 persons (17%)
Hypoalphalipoproteinemia	51 persons (35%)
Hypertension	123 persons (84%)
Family history positive for cardiovascular disease	122 persons (83%)
Diabetes mellitus	29 persons (20%)
Framingham Risk Score (10-year risk)	13 (9 – 22) %
SCORE (10-year risk)	5 (2 – 10) %
C-reactive Protein	2.8 (1.3 – 5.7) mg.l^{-1}
Leukocyte count	5.1 × 10^9 l^{-1} (5.9 × 10^9 – 7.0 × 10^9) l^{-1}
Creatinine	0.93 ± 0.20 mg·dl^{-1}
Creatinine > 1.2 mg·dl^{-1}	12 persons (8%)
Uric acid	(5.89 ± 1.45) mg·dl^{-1}
Fasting glucose	86 (79 – 97) mg·dl^{-1}
Systolic blood pressure	(146 ± 19) mmHg
Diastolic blood pressure	(82 ± 9) mmHg
Total/HDL-cholesterol ratio	4.5 ± 1.2
Triglycerides	(137 ± 71) mg·dl^{-1}
LDL-cholesterol	(130 ± 42) mg·dl^{-1}
HDL-cholesterol	(48 ± 14) mg·dl^{-1}
Skin autofluorescence (arm)	(2.16 ± 0.41) × 10^2 AU
Mean IMT CCA	0.71 (0.65 – 0.82) mm
Mean IMT ICA	0.79 (0.66 – 1.11) mm (n = 119)
Mean IMT CB	1.06 (0.87 – 1.33) mm (n = 130)

Values are means ± standard deviation, median with interquartile range, or number/percentage of subjects.
HDL – high-density lipoprotein, LDL – low-density lipoprotein, AU – arbitrary units, IMT – intima media thickness, CCA – common carotid artery, ICA – internal carotid artery, CB – carotid bulb.

Tab. 2. Food and drinking habits of the 147 participants.

Food and drinking habit	Value
Drinking wine	42 (29%)
If yes, mean glasses of wine a week	2.9 ± 1.9
Drinking beer	25 (17%)
If yes, mean glasses of beer a week	4.3 ± 3.2
Drinking alcohol	66 (45%)
If yes, mean glasses of alcoholic drinks a week	3.6 ± 3.0
Drinking milk	104 (71%)
If yes, mean glasses of milk a week	3.1 ± 1.5
Drinking tea	105 (71%)
If yes, mean cups of tea a day	2.3 ± 1.5
Drinking coffee	141 (96%)
If yes, cups of coffee a day	3.4 ± 2.2
Consumption of meat	145 (99%)
If yes, pieces of meat a week	5.1 ± 1.5
Consumption of eggs	127 (86%)
If yes, eggs a week	2.3 ± 2.8
Consumption of fish	100 (68%)
If yes, pieces of fish a week	1.5 ± 0.6
Consumption of fruits	139 (95%)
If yes, pieces of fruits a week	2.1 ± 0.9
Usage of seed oil	8 (5%)
Usage of olive oil	36 (25%)
Usage of margarine	87 (59%)
Usage of butter	4 (3%)
No usage of fat	11 (8%)

Values are means ± standard deviation, or number/percentage of subjects.

correlations of non-normally distributed data, we used the Spearman's rho test and for parametric data the Pearson's test. Multivariable stepwise regression analysis was used to determine independent effects on skin AF and IMT. To show medians, interquartile ranges and outliers, box plots were used. A two-side p value less than 0.05 was considered statistically significant. Data are shown as means ± standard deviation or medians and interquartile ranges. SPSS software, version 15.0.1 (SPSS, Chicago, Illinois, USA) was used for statistical analyses.

RESULTS

Patient characteristics

From the initial study population of 168 subjects, in whom we measured skin AF at the arm four times, 21 were excluded because of missing data. The characteristics of these excluded subjects were similar to the study group. Clinical characteristics as well as the food and drinking habits of the remaining participants are summarized in Tab. 1 and Tab. 2, respectively. We obtained the three-day food diaries from 73 subjects of the 147 participants.

Food and drinking habits, skin AF and IMT

We found a lower skin AF ($p = 0.042$) and lower mean IMT of the CCA ($p = 0.007$) in the subjects who consumed wine (Fig. 1a-b). No differences in mean IMT of CB or ICA were found between wine drinkers and the other subjects. The amount of wine did not make a difference.

Since a small number of patients use predominately sunflower oil or butter, we divided the predominant use of fat in the two categories: olive or sunflower oil versus margarine or butter usage. We found a higher skin AF in the margarine-butter group ($p = 0.018$; Fig. 2), but we did not find a difference in any IMT value. Predominant use of olive
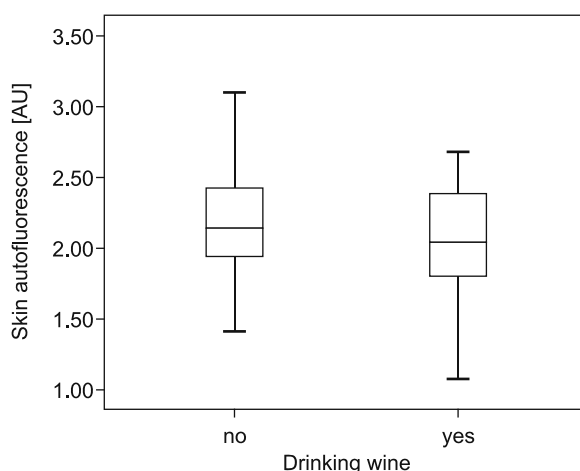
Fig. 1a. Box plot of skin autofluorescence in arbitrary units (AU) and consumption of wine.
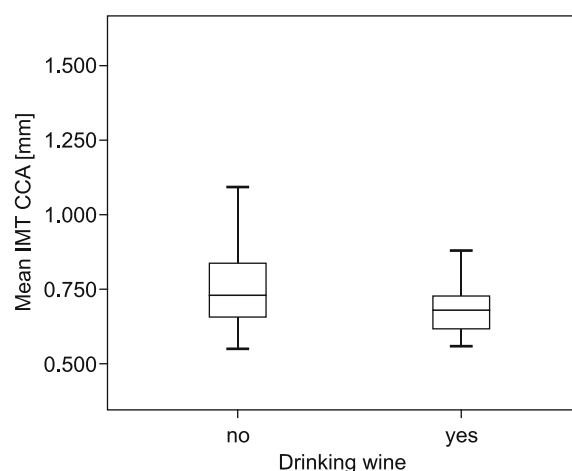
Fig. 1b. Box plot of intima media thickness of the common carotid artery (in mm) and consumption of wine.
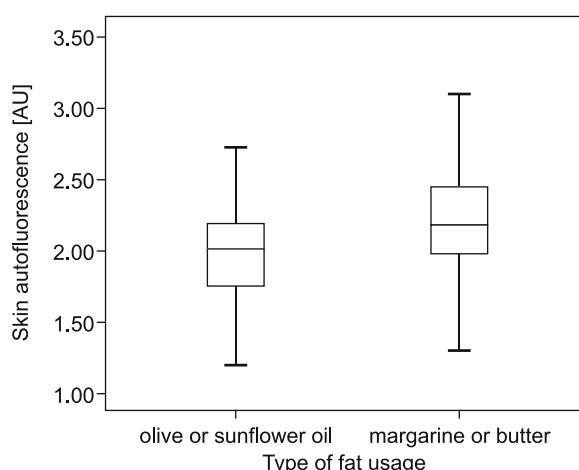
Fig. 2. Box plot of skin autofluorescence in arbitrary units (AU) and type of fat usage.

oil negatively related with skin AF ($p = 0.018$). All other food and drinking habits (consumption of beer, spirits, alcohol, eggs, meat, fish, milk or coffee) did not correlate with the results of the skin AF or mean results of IMT measurements.

Concerning the data from the three-day food diaries, the mean AGE intake calculated from the Goldberg tables did not relate with skin AF and any of the mean IMT values. Negative relations were only found between skin AF and mean intake of energy ($r = -0.256$; $p = 0.029$), proteins ($r = -0.329$; $p = 0.004$) and saccharides ($r = -0.272$; $p = 0.020$). Multiple regression showed that skin AF was independently and negatively related with mean protein intake ($R^2 = -0.108$; $p = 0.004$), and that IMT of CB was independently related to unsaturated fats ($R^2 = 0.062$; $p = 0.043$). Positive correlations were found between cardiovascular

risk factors and IMT, and cardiovascular risk factors and skin AF (Tab. 3). No correlations were found between mean IMT CCA, mean IMT ICA and mean IMT CB and skin AF.

DISCUSSION

This study shows that, in elderly patients with moderately increased cardiovascular risk, consumption of wine is associated with discernible positive differences in both skin AF and IMT. Concerning the dominant type of fat used, skin AF was higher in those mainly consuming butter or margarine. No relation was found between other variations in food habits and skin AF or IMT. In particular, no relation was found between the intake of AGE and skin AF in the subgroup, in which this intake was estimated based on three-day questionnaires. The negative relation between the use of (red) wine and AGE formation has been addressed earlier [41]. However, this is the first time that support is found in an in vivo situation in humans for a relation between wine consumption and tissue accumulation of AGE, as estimated by skin AF. Moreover, this association also extends to a lower IMT, which suggests a beneficial effect on early atherosclerosis. Wine contains polyphenols with an antioxidant activity [42, 43], which may potentially reduce the AGE formation induced by oxidative stress [44].

We could not confirm the published inverse relation between mean IMT CCA and olive oil consumption [45]. However, an inverse relation between the main intake of olive oil and skin AF was observed.

Tab. 3. Univariate significant correlations of cardiovascular risk factors and intima media thickness, as well as cardiovascular risk factors and skin autofluorescence.

Cardiovascular risk factors	IMT			Skin autofluorescence
	CCA	ICA	CB	arm
Age	$r = 0.362$; $p < 0.001$	N. S.	$r = 0.306$; $p < 0.000$	$r = 0.307$; $p < 0.001$
Body mass index	N. S.	N. S.	N. S.	$r = 0.199$; $p = 0.016$
Framingham risk score	$r = 0.241$; $p = 0.003$	$r = 0.230$; $p = 0.012$	$r = 0.287$; $p = 0.001$	$r = 0.245$; $p = 0.003$
SCORE risk score [a]	$r = 0.282$; $p < 0.001$	$r = 0.232$; $p = 0.011$	$r = 0.334$; $p = 0.000$	$r = 0.363$; $p < 0.001$
Leukocyte count	N. S.	N. S.	N. S.	$r = 0.233$; $p = 0.005$
Fasting glucose	N. S.	N. S.	N. S.	$r = 0.214$; $p = 0.010$
Systolic blood pressure	$r = 0.208$; $p = 0.012$	$r = 0.218$; $p = 0.017$	$r = 0.266$; $p = 0.002$	$r = 0.238$; $p = 0.004$
Uric acid	$r = 0.172$; $p = 0.037$	$r = 0.234$; $p = 0.011$	N. S.	N. S.
HDL-cholesterol	N. S.	N. S.	$r = -0.246$; $p = 0.005$	N. S.

a – assessed by using the chart for populations at low cardiovascular disease risk.
r – correlation coefficient, p – statsitical significance, N. S. – not significant, IMT – intima media thickness, CCA – common carotid artery, ICA – internal carotid artery, CB – carotid bulb.

By stepwise multiple regression analysis, we found an inverse relation between skin AF and the mean intake of proteins. If subjects eat less saccharides, they probably eat more proteins and fat, both richer in AGE. However, this is not in line with the finding of negative correlation between protein intake and skin AF and the lack of relation between the total amount of high-AGE-containing fats and skin AF. The way of processing and type of fat may be more important than the total amount of fat. For our food diary, we used the information of the Netherlands Nutrition Centre. We calculated from the obtained three-day food diary the AGE intake using the Goldberg tables. Remarkably, we did not find any correlation between skin AF and exogenously derived AGE, calculated from the food diary [8]. The biological pathway from exogenously derived AGE to skin collagen is long. On the other hand, a recent study on humans showed a significant increase in plasma AGE levels within two hours following an AGE-rich meal. Thirdly, AGE ingested with food may be poorly absorbed. The estimation of oral bioavailability is reported differently, ranging from approximately 2% to 60% [4, 46, 47]. Obviously, the absence of a relation between skin AF and AGE obtained from food may also be due to limitations in the use of the three-day questionnaire, because it may have lacked the ability to quantify AGE content in food with sufficient accuracy. Moreover, the AGE content of commonly consumed foods might be given imprecisely as the enzyme-linked immunosorbent assay based on an anti-*N*-carboxymethyllysine antibody as used by GOLDBERG et al. [8] might be considered as not fully appropriate for determination of carboxymethyllysine in foods. However, no other method to calculate the AGE content of foods is available. Finally, it is possible that, in this (sub)group of persons with a relatively well-preserved renal function, the effects of even larger variations in exogenous AGE supply do not affect skin AF or IMT. The consequences of changes in exogenous AGE supply on its accumulation in plasma and even more in the tissues, and ensuing vascular damage, strongly depend on renal function and may only be evident or relevant in persons with renal failure [17]. Additionally, the Goldberg questionnaire was originally developed for use in USA and has been translated to the Dutch situation. It is generally accepted that the eating habits may differ between countries and therefore the questionnaire may not be suited for a population in the Netherlands. This implies that before drawing definite conclusions, a questionnaire should be developed, taking into account the Dutch eating habits.

In conclusion, we demonstrated that consumption of wine and a diet consisting of high amounts of proteins together with low amounts of saturated fats is associated with lower values of skin AF. Since skin AF is considered to be a non-invasive marker for AGE, these dietary habits may result in a decreased tissue accumulation of AGE. According to the current concept AGE are involved in the progression of atherosclerosis and hence the development of CVD. Unhealthy dietary habits may induce increased AGE formation in tissues and may be a source for exogenously derived AGE itself [48]. We could not demonstrate a direct relation between exogenous AGE intake and skin AF in this study, probably due to methodological issues. Further research is needed to address this issue.

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