

Non-invasive cardiovascular risk assessment

The AGE Reader provides an immediate prediction for the cardiovascular risk of your patient. The non-invasive and extensively validated AGE Reader is and ideal tool for point of care testing. The measurement result is available in 12 seconds and can be exported directly.



A perfect tool for diabetologists and family doctors.

About AGEs

(Advanced Glycation Endproducts)

AGEs are the result of a chain of chemical reactions (the Maillard reaction) after an initial glycation. AGEs normally accumulate slowly over a person's lifetime in tissues with slow turnover. But this process occurs more rapidly in patients with conditions such as diabetes mellitus, renal failure and cardiovascular disease. AGEs also accumulate rapidly under circumstances of oxidative stress. These accumulated AGEs play a key role in the development of diabetes and its complications. The level of AGEs in tissue reflects the glycometabolic memory and is a valuable predictor of cardiovascular events and (pre)diabetes.



AGEs play a key role in the pathogenesis of many age-related diseases, such as diabetes, cardiovascular disease and renal failure.

Measuring **AGEs**

With any other measurement it has been complicated to measure tissue AGEs in patients because they are expensive, time consuming, lack specificity, are poorly reproducible and/or are invasive. The AGE Reader is the answer to the need for measuring AGEs without the disadvantages of the existing methods. This state of the art device provides a simple non-invasive solution, which allows clinicians to determine the AGE level within 12 seconds. Many advanced glycation endproducts (AGEs) have a characteristic fluorescence. Moreover, tissue fluorescence in (invasive) biopsies has an established association with chronic complications. The AGE Reader is able to easily, quickly and noninvasively measure AGEs by means of fluorescence techniques¹.



AGE Reader

The AGE Reader provides an immediate prediction for cardiovascular risk. The measurement is reliable, real time, non-invasive and easy to use.

Moreover, the AGE Reader has been validated in clinical studies around the world. The AGE Reader has been used in clinical practice and research since 2006 in over 1000 clinics worldwide. Since the introduction of the AGE Reader more than 200 peer reviewed papers have been published. These papers give an overview of clinical studies in diabetes², cardiovascular disease³ and renal disease⁴.





Clinical validation

Key conclusions of the clinical validation studies using the AGE Reader:

- Reflects vascular damage in the diabetes patients².
- Identifies diabetic patients at risk of developing (cardiovascular and microvascular) complications^{5,6,7}.
- Predicts the risk of having or developing diabetes and the metabolic syndrome^{8,9}.
- Strong predictor of major cardiovascular events in peripheral artery disease¹⁰.

Clinical use

Clinical professionals have been successfully using the AGE Reader in their clinics for over 10 years. The AGE Reader is the answer to the need to quickly, reliably and non-invasively measure the cardiovascular risk of your patient.

The AGE Reader assists clinical professionals in identifying patients with an increased cardiovascular risk. This helps clinicians to decide whether a change in treatment is needed. This non-invasive and convenient measurement can be performed by any clinical professional and is completed within 12 seconds.

The AGE Reader App

Export the measurement directly to the AGE Reader App using the Bluetooth connection and add other cardiovascular risk factors to generate a comprehensive Cardiovascular Risk Report. For each patient all visits can be documented and consulted in the easy to use App. The Cardiovascular Risk Report can simply be **printed, saved** and **shared**.

Download the AGE Reader App free of charge on www.diagnoptics.com. Available for:

	X
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Explain & Motivate patients

The graphical display allows you to combine the AGE Reader result with desired other cardiovascular risk factors and explain the results to patients. A hardcopy of the report can be handed to the patient, which will make it easier to understand the measurement result and the associated cardiovascular risk.

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AGEs DamAGE Our Bodies. (Advanced glycation endproducts)



By <u>Brady Hartman</u> in <u>Anti-Aging Science</u>, <u>Healthy Living</u> October 19, 2017 Sharing is caring!

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Reading Time: 11 minutes. >>

Summary: AGEs (advanced glycation end-products) are in the spotlight again as <u>geroscientists</u> implicate this toxic waste of our bodies in a multitude of health conditions, including <u>inflammaging</u>, cancer, and <u>diabetes</u>. Some scientists go as far as to link AGEs to an increase in the <u>chronic inflammation which leads to heart</u> <u>attacks</u>. [This article first appeared on the website <u>LongevityFacts.com</u>. Author: <u>Brady Hartman</u>.]

Executive Summary

Are advanced glycation end-products (AGEs) a villain that causes chronic diseases or are they merely an innocent bystander?

Anti-aging scientists are coming to the growing conclusion that AGEs play a significant role in inflammation and the chronic diseases of aging, such as diabetes, heart disease, and many others. Furthermore, some geroscientists suspect that AGEs significantly contribute to cancer.

In an earlier review that links AGEs with cancer, David P. Turner, a researcher with the Department of Pathology & Laboratory Medicine at the Medical University of South Carolina in Charleston (MUSC) sums it up by saying,

"Advanced glycation end-products (AGEs) are reactive metabolites produced as a by-product of sugar metabolism. Failure to remove these highly reactive metabolites can lead to protein damage, aberrant cell signaling, increased stress responses, and decreased genetic fidelity."

Earlier this year, Turner updated his research on the link between advanced glycation end-products and cancer, as you will read later on.

Turner and other researchers are adding to the growing body of evidence against AGEs. Geroscientists are sure that the steps we take to reduce AGEs are incredibly good for our health. That is, having low blood sugar, low cholesterol levels and eating less processed foods are sure-fire ways to lengthen our lives and coincidently also reduce advanced glycation end-products.

Bottom Line: AGEs are bad for our health. However, scientists don't know the exact extent. There is hope, however. Scientists have come up with ways to prevent the build-up of advanced glycation end-products.

AGEs DamAGE Our Bodies

What Scientists Know About AGEs

Turner is not the only <u>geroscientist</u> accusing AGEs of misconduct. Other researchers already know that advanced glycation end products naturally build up in our bodies as we grow older, although the things we eat can also increase their levels. Geroscientists show that AGEs raise the level of cellular damage, which in turn increases the levels of chronic inflammation. Aging is accompanied by a <u>chronic low-level inflammation</u> <u>called inflammaging</u> that affects nearly every cell in the body. Moreover, as you will read later on, they have the evidence to back it up. As well, leading geroscientists, such as Carlos Lopez-Otin, the author of the landmark report *The Hallmarks of Aging*,

believe that the chronic inflammation significantly contributes to the progression of a slew of chronic diseases.

Strong Suspicions About AGEs

Many researchers have indicted AGEs as a major villain in chronic diseases with an inflammatory component such as asthma, atherosclerosis, arthritis, cancer, heart disease, kidney disease, blindness, and neuropathic pain. While geroscientists are confident that advanced glycation end products increase levels of chronic inflammation, and contribute to a handful of chronic diseases, they are not quite sure how large of a role AGEs play in these conditions.

Bad habits such as a high-fat, high-sugar diet simultaneously lead to both increased incidences of these chronic diseases as well as increased levels of advanced glycation end products. Whenever any one of these chronic diseases strikes, large numbers of AGEs are always at the scene of the crime.

Bottom Line: There is a growing consensus that AGEs are responsible for vandalism to our bodies, increase inflammaging and stoke the fires of chronic diseases.



glycation end-products form (AGEs). Credit: David Turner (2017)

It's So Easy to Get Glycated These Days

AGEs are a diverse array of compounds produced in the body through a variety of chemical reactions. In a process called glycation, AGEs form when sugars combine with proteins or fats. When sugar latches on to one of these molecules, it creates what is known as an advanced glycation end-product.

For example, consider a lab test called the hemoglobin A1c test. If you have ever been tested for diabetes or prediabetes, then you are probably aware of the A1c test. This common diagnostic for diabetes measures the blood levels of an advanced glycation end-product called *glycated hemoglobin*. As an aside, if you are over 45 or overweight, the ADA says you ought to get tested for <u>prediabetes</u> and <u>diabetes</u>.

The A1C test also abbreviated HbA1c, and HbgA1c is a blood test that measures a person's average blood sugar levels over the past three months. To keep us even more confused, doctors use a multitude of names, such as glycohemoglobin, glycated hemoglobin, and glycosylated hemoglobin to all refer to the same compound.

Whatever you want to call it, glycated hemoglobin is a common AGE in your bloodstream. Glycosylated hemoglobin forms when sugar chemically attaches to hemoglobin. The more sugar in your blood, the more glycated hemoglobin, and the higher your A1c reading. The higher your A1c level, the more likely your doctor will diagnose you with prediabetes or diabetes.

What Increases Advanced Glycation End-Products?

AGEs are more likely to form when blood sugar is elevated, which explains the higher levels of advanced glycation end-products in people with poorly controlled type 2 diabetes. People also consume advanced glycation end-products, mostly from cooked or processed foods, and absorb them from tobacco smoke. The body can eliminate AGEs using enzymes and expel them via the kidneys, but the advanced glycation endproducts formed in the body are more likely to accumulate.



With a toaster, anyone can make advanced glycation end-products (AGEs) at home. Just brown the crust. Credit: StockSnap

Who Discovered AGEs?

In 1912 a French scientist named Louis-Camille Maillard discovered how AGEs form in food. Maillard was the first to explain the chemical reaction – subsequently named the Maillard reaction – results from the browning of certain foods when cooked, such as bread crust and meat. Scientists have researched AGEs for the past 20 years, and are increasingly focused on food sources of these compounds.

Why Do Advanced Glycation End-Products Form?

Advanced glycation end-products build up naturally in the body as we grow older. While high blood sugar is the main culprit, high cholesterol also accelerates the build of AGEs. We get advanced glycation end-products through our diet, as well. Components of certain foods called dietary advanced glycation end-products (dAGEs) contribute to the levels of AGEs in our body and also increase the risk of diseases such as kidney disease and atherosclerosis. Scientists do not fully understand how well the AGEs we consume from foods are absorbed by the gut, nor whether vitamins and other nutrients influence their effects on the body.

Advanced Glycation End-Products and Diabetes

Scientists strongly suspect that advanced glycation end-products are involved in the progression of diabetes and the complications of the disease.

In diabetics, uncontrolled blood sugar leads to higher risks of all kinds of complications, including heart disease, stroke, kidney disease, amputations, and blindness. Scientists have not conclusively fingered AGEs as the culprit behind these complications, but in my book, they are the number one suspect.

Research on Advanced Glycation End-Products

The strongest research evidence studies the effects of AGEs in humans. However, a research experiment in which scientists fed a diet high in AGEs to mice is very telling.



Lab animals are easier to test AGEs on because they develop diseases more quickly. Credit: Tibor Janosi Mozes

Dietary AGEs Induce Diabetes in Mice

While human studies are far more reliable, mouse studies can add insight because researchers can do things to mice that would be unethical to humans. Furthermore, mice develop diseases much faster than humans, who take decades to develop chronic disease. For example, in a study published in *Diabetes*, the journal of the American Diabetes Association, a team of researchers at New York's Mount Sinai Medical Center (MSMC) fed lab mice a diet high in AGEs and compared them to normally fed controls. The MSMC researchers found that the mice on a diet high in dietary AGEs were significantly more like to develop diabetes when compared to controls. The researchers divided the mice into three groups: a) a high-fat diet high in AGEs b) highfat diet low in AGEs c) normally fed control mice. After waiting for six months, the <u>human equivalent</u> of 34 years, 75% of the mice on the high AGE diet developed diabetes, while none of the mice on the low AGE diet developed the condition. The mice on the two high-fat diets gained similar amounts of body weight.

Bottom Line: Researchers showed that, at least for mice, fat is not to blame for causing diabetes, instead of advanced glycation end-products are responsible.



Testing AGEs (advanced glycation end-products) in people gives the best results. Credit: RawPixel

Human Study On Advanced Glycation End-Products

In a study published in the journal Diabetologia in 2016, researchers at New York's Mount Sinai Medical Center (MSMC) analyzed the role of AGEs and the risk of type 2 diabetes. The MSMC researchers randomized 61 obese people with metabolic syndrome into two groups: Half ate a diet low in AGEs while the other half ate a standard American diet that was high in advanced glycation end-products. The participants on the low-AGE diet were told to avoid grilling, baking, or frying food to reduce consumption of advanced glycation end-products. After one year, the group on the low AGE diet had lower blood levels of advanced glycation end-products, a reduction in insulin resistance, a decrease in markers of inflammation and oxidative stress, and a small amount of weight loss to boot.

AGEs and Insulin Sensitivity in Humans

Similarly, in a small Australian study published in the American Journal of Clinical Nutrition in 2016, healthy overweight people were put on a low AGE diet low for two weeks. At the end of the study period, the participants on the diet low in AGEs had improved insulin sensitivity compared to those on a diet high in advanced glycation end-products.



Do AGEs cause diabetes? The evidence against advanced glycation end-products is compelling. Credit: Tesa Robbins.

Do AGEs Cause Diabetes?

The levels of advanced glycation end-products in our bodies increase as we age, and the rates of diabetes rise correspondingly. In fact, by the age of 65, two-thirds of all American adults have either diabetes or prediabetes. Correlation does not imply causation, but it sure is a hint. The correlation combined with the animal and human research studies strongly argue that AGEs make us significantly more susceptible to prediabetes and <u>type 2 diabetes</u>.

<u>Prediabetes</u> is the condition where a person has high blood sugar, but not high enough to be considered diabetes. The World Health Organization (WHO) <u>estimates that</u> <u>prediabetes</u> kills one and half times as many people as diabetes because it is far more prevalent and far more undetected than diabetes.

This is a great tragedy due to that fact that prediabetes can be treated by a diet and exercise, or medication. Coincidentally, the drug <u>metformin</u>, the same medication that doctors use to treat prediabetes and diabetes is also a lifespan-extension drug.

Cardiovascular Disease of the AGEs

Scientists strongly suspect that AGEs stoke the disease of atherosclerosis. In turn, atherosclerosis, also called hardening of the arteries, leads to cardiovascular disease and heart attacks. An earlier report links <u>inflammation to heart disease</u>.

Cancer of the AGEs

Researcher David Turner has sharpened his pencil as of late. This year (2017) Turner published an updated review of the role of AGEs in cancer in the journal Advances in Cancer Research. In this special compendium on cancer, which includes the works of many other researchers, Turner makes a stronger case for a role of AGEs in cancer, saying

"Due to their links with lifestyle and the activation of disease-associated pathways, AGEs may represent both a biological consequence and a biobehavioral indicator of poor lifestyle which may be targeted within specific populations to reduce disparities in cancer incidence and mortality."

AGEs in Alzheimer's Disease

As we grow older, AGEs accumulate in the brain and other tissues. Researchers have implicated advanced glycation end-products in cultured neuronal toxicity as well as beta-amyloid aggregation in Alzheimer's disease.

Cleaning out Advanced Glycation End-Products

Medical science has yet to invent a magic pill or potion that cleans a significant portion of AGEs from the body. Prevention is your best bet, and keeping your blood sugar low is a proven way to prevent a ton of chronic diseases, including cancer, heart disease, and diabetes.

For example, consider glucosepane, by far the most common AGE in human tissue. <u>Glucosepane forms cross-links</u> in the collagen of our arteries and joints, causing them to become stiffer. As we age, the level of glucosepane in our collagen increases, leading to arteriosclerosis and joint stiffening. Due to decades of elevated blood sugar levels, this effect is accentuated in people with diabetes.

To reverse aging, geroscientists are searching for compounds that reverse cross-linking and return our joints and arteries to their youthful, supple state. Researchers that they had found such a substance with the discovery of a compound called alagebrium (ALT-711.)

ALT-711 produced seemingly miraculous effects in aging and diabetic lab animals and substantially lowered their blood pressure. However, alagebrium was a disappointment when tested on humans, producing only minor effects.

If we are to reverse aging, research on glucosepane is sorely lacking and sorely needed. Reason at <u>fightaging.org has a good description</u> of the subject, as well as a <u>recent update on the efforts of researchers</u> developing monoclonal antibodies that attack glucosepane.

Bottom Line: There are no adequate cleaning *products* to get rid of advanced glycation *end-products*.



All-beef patty and AGEs on a sesame seed bun. Advanced glycation end-products are highest in meat, especially flame-broiled. Credit: Robert Owen-Wahl.

Reducing AGEs in Food

AGEs occur naturally in many foods, especially animal-derived products, such as meats that are rich in fat and protein. The level of advanced glycation end-products in food depends on how you cook or prepare it. High temperatures produce more advanced glycation end-products, but AGEs can also be found in aged cheeses (no pun intended). As well, specific processes used by food manufacturers to add flavor or color to their products also boost advanced glycation end-products. Compared to low-heat methods, more advanced glycation end-products form when cook meat with dry-heat methods, including roasting, grilling, frying, or baking. All of these methods cook at relatively high temperatures and for longer times than the low-heat methods. Low-heat methods produce the lowest levels of AGEs and include boiling, steaming, poaching, or stewing. As well, low-heat methods retain food moisture. Microwaving produces fewer advanced glycation end-products because of its relatively short cooking times. The source of animal protein also affects the generation of AGEs, with beef having the most, and fish the least. The fat content in meat tends to contain more advanced glycation end-products. Marinating meat with acidic preparations such as lemon juice and vinegar inhibits advanced glycation end-product formation.

On the flip side, <u>fruits and vegetables are not only healthy</u>, but they are also low in AGEs, even when cooked. As well, many grains are lower in advanced glycation end-products. However, grain products such as cookies, crackers, and chips are higher in AGEs because they are processed with dry heat methods and loaded with additional fats and sugars. Despite this, when compared to meat, processed grain products such as potato chips have a lower content of advanced glycation end-products. <u>Potatoes are a healthy vegetable</u>, unfortunately, frying them boosts their levels of AGEs.

Bottom Line

AGEs are bad for our health. However, scientists do not know the precise extent. Geroscientists need to perform more research to fully understand the health effects of advanced glycation end-products, including both those produced by the body and those we consume from foods. Meanwhile, what we know about AGEs does not change our thinking about healthy eating. Recent research reports have given us good reasons to limit fatty meats, especially those cooked with high heat, a process that produces carcinogens such as heterocyclic amines. As well, doctors advise us to limit fried or highly processed foods such as French fries and chips. Moreover, so-called hearthealthy eating patterns, such as the Mediterranean diet, are low in advanced glycation end-products. Also, low AGEs diets are those low in processed foods, meat and fast foods and rich in fruits and vegetables, legumes, whole grains, fish, and low-fat dairy.

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RESEARCH

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Skin autofluorescence, a non-invasive biomarker for advanced glycation end products, is associated with the metabolic syndrome and its individual components

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Abstract

Background: The metabolic syndrome (MetS) comprises several cardiometabolic risk factors associated with increased risk for both type 2 diabetes and cardiovascular disease. Skin autofluorescence (SAF), a non-invasive biomarker of advanced glycation end products accumulation, is associated with cardiovascular complications in subjects with diabetes. The aim of the present study was to examine the association between SAF and the presence of MetS as well as its individual components in a general population.

Methods: For this cross-sectional analysis, we included 78,671 non-diabetic subjects between 18 and 80 years of age who participated in the LifeLines Cohort Study and had SAF measurement obtained non-invasively using the AGE Reader. MetS was defined according to the revised NCEP ATP III criteria. Students unpaired t test was used to test differences between groups. Both logistic and linear regression analyses were performed in order to test associations between the individual MetS components and SAF.

Results: Subjects with MetS had higher SAF (2.07 ± 0.45 arbitrary units, AU) compared to individuals without MetS (1.89 \pm 0.42 AU) (p < 0.001). There was a positive association between the number of MetS components and higher SAF Z-scores (p < 0.001). Individuals in the highest SAF tertile had a higher presence of MetS (OR 2.61; 95% CI 2.48–2.75) and some of the individual components compared to subjects in the lowest SAF tertile. After correction for age, gender, creatinine clearance, HbA1c and smoking status, only elevated blood pressure and low HDL cholesterol remained significantly associated with higher SAF (p = 0.002 and p = 0.001 respectively).

Conclusion: Skin autofluorescence was associated with the presence of MetS and some of its individual components. In addition, increasing SAF Z-scores were observed with a higher number of MetS components. Prospective studies are needed to establish whether SAF can be used as an (additional) screening tool to predict both cardiovascular disease and type 2 diabetes in high-risk populations.

Background

Advanced glycation end products (AGEs) comprise a group of largely irreversibly glycated proteins, lipids and

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impaired renal excretion in subjects with kidney failure, as well as dietary intake and tobacco smoking [5-8].

Since the past decade, it has become possible to estimate tissue AGE accumulation non-invasively by measuring autofluorescence of the skin (SAF) [9]. In our previous study we showed that SAF was associated with several clinical and lifestyle parameters [10]. SAF has previously been validated against tissue AGE measurements and reference values against age were obtained [11, 12]. SAF was reported to be elevated in subjects with type 1 and 2 diabetes [9, 13]. Moreover, SAF has been shown to be a strong predictor of long-term cardiovascular complications and mortality in both type 1 and type 2 diabetes and end-stage renal failure [14-18]. In addition, recent studies have shown higher SAF levels to be associated with coronary artery disease, peripheral artery disease and (sub)clinical atherosclerosis independent of diabetes [19–21].

The metabolic syndrome (MetS) is a cluster of cardiometabolic abnormalities associated with increased risk for cardiovascular disease (CVD) and type 2 diabetes mellitus [22, 23]. The MetS is a worldwide problem which prevalence increases worldwide, particularly due to the growing epidemic of obesity [24–26]. Glycation is known to play an essential role in the mechanism that leads to the formation of AGEs [6].

It has been shown that AGEs increase inflammation and oxidative stress hereby promoting insulin resistance. On the other hand, a low AGEs diet improves insulin sensitivity [27, 28].

However, the association of the other cardiometabolic components with SAF has not been assessed in detail. As SAF measurement might be used as a future screening tool in high-risk populations, such as the MetS, to refine estimation of risk of future cardiovascular events or development of type 2 diabetes, knowledge of potential associations with SAF is important. Therefore, the aim of this cross-sectional study was to assess SAF in subjects with MetS. We examined the association between the individual MetS components and SAF in a large-scale general population.

Methods

Participants

Subjects included were participants from the LifeLines Cohort Study [29], a large population-based cohort study in the northern region of the Netherlands examining the interaction between genetic and environmental factors associated with chronic diseases and healthy ageing. Between 2006 and 2013, individuals from the three northern provinces of the Netherlands were invited to participate in the study. At baseline, both physical examination and extensive questionnaires were collected from more than 167,000 participants [30]. All participants have provided written informed consent before participating in the study. The study has been approved by the Medical Ethics Review Committee of the University Medical Center Groningen. For the present study, we included subjects of Western European descent between 18 and 80 years of age having a SAF measurement available (n = 82,515). We excluded subjects with either missing data for MetS status (n = 1221) and those with a serum creatinine >140 μ mol/L (n = 75), as severely reduced kidney function itself increases AGE levels. Furthermore, subjects with missing data for diabetes status (n = 86) were excluded, as were those with type 1 diabetes (n = 177), type 2 diabetes (n = 2157) or previous gestational diabetes (n = 128) leaving 78,671 non-diabetic individuals for analyses.

Clinical and lifestyle data

The following clinical data were used: age, gender, body mass index (BMI), waist circumference, systolic and diastolic blood pressure, serum lipids, fasting plasma glucose, HbA1c, creatinine clearance, use of medication, and selfreported history of CVD (myocardial infarction and cerebrovascular accident). Information regarding smoking behaviour was collected by extensive questionnaires, as described earlier [10]. Pack-years of smoking were calculated as the number of cigarette packages smoked per day multiplied by the number of years an individual had smoked. Data regarding coffee consumption (cups of coffee per day) were obtained by questionnaire. We were not able to distinguish between caffeinated and decaffeinated coffee consumption.

Physical measurements

Weight was measured to the nearest 0.1 kg and height and waist circumference to the nearest 0.5 cm by trained technicians using calibrated measuring equipment, with participants wearing light clothing and no shoes. Waist circumference was measured with a tape around the body between the lower rib margin and the iliac crest. BMI was calculated as weight divided by height squared (kg/m^2) . Systolic and diastolic blood pressure were measured every minute during 10 min in the supine position using an automated Dinamap Monitor (GE Healthcare, Freiburg, Germany). The average of the last three readings was recorded. SAF was assessed as the mean of three consecutive measurements using the AGE Reader (DiagnOptics Technologies BV, Groningen, the Netherlands) in all participants, as described previously [9–11].

Biochemical measures

Blood samples were taken in the fasting state between 8.00 and 10.00 a.m. and transported to the LifeLines laboratory facility at room temperature or at 4 °C, depending on the sample requirements. On the day of collection, HbA1c (EDTA-anticoagulated) was analyzed using a NGSP-certified turbidimetric inhibition immunoassay on a Cobas Integra 800 CTS analyzer (Roche Diagnostics Nederland BV, Almere, the Netherlands). Serum creatinine was measured on a Roche Modular P chemistry analyzer (Roche, Basel Switzerland), and creatinine clearance was calculated with the chronic kidney disease epidemiology collaboration (CKD-EPI) formula [31]. Total and high density lipoprotein (HDL) cholesterol were measured using an enzymatic colorimetric method, triglycerides using a colorimetric UV method, and low density lipoprotein (LDL) cholesterol using an enzymatic method, on a Roche Modular P chemistry analyzer (Roche, Basel, Switzerland). Fasting blood glucose was measured using a hexokinase method.

Definition of the metabolic syndrome

Diagnosis of MetS was established if a subject satisfied at least three out of five criteria according to the revised National Cholesterol Education Programs Adults Treatment Panel III (NCEP ATPIII criteria) [22]: (1) systolic blood pressure \geq 130 mmHg and/or diastolic blood pressure \geq 85 mmHg and/or use of antihypertensive medication; (2) HDL cholesterol levels <1.03 mmol/L in men and <1.30 mmol/L in women and/or use of lipid-lowering medication influencing HDL levels; (3) triglyceride levels \geq 1.70 mmol/L and/or use of triglyceride-lowering medication; (4) waist circumference \geq 102 cm in men and \geq 88 cm in women; (5) fasting glucose level between 5.6 and 7.0 mmol/L. As mentioned earlier, people with diabetes were excluded from analysis to reflect 'true' MetS.

Calculations and statistical analyses

Data are shown as mean \pm standard deviation (SD) or median and interquartile range (IQR) in case of nonnormally distributed data. Student's unpaired *t* test, Analysis of Variance (ANOVA) or Chi Square test were performed to compare groups. Age-adjusted SAF levels (Z-scores) were calculated based on the total population (separated for males and females) as SAF and MetS are strongly affect by ageing [10, 32]. Next, we classified individuals into tertiles of their age-adjusted SAF Z-scores, in men: lowest SAF Z \leq -0.59, intermediate SAF -0.60 < Z < 0.49 and highest SAF Z \geq 0.50; in women: lowest SAF Z \leq -0.72, intermediate SAF -0.73 < Z < 0.33 and highest SAF Z \geq 0.34. Logistic regression analysis was performed to assess whether either of the SAF groups (low SAF was set as a reference) were associated with the presence of MetS and its components. Model 1 shows the unadjusted association between tertiles of SAF Z-scores and MetS as well as its individual components. In model 2, we adjusted for gender and BMI. In model 3, we additionally adjusted for creatinine clearance, HbA1c, current smoking, pack-years and CVD history. Linear regression analysis was performed to examine the association between each of the individual MetS components and SAF among the population with MetS. In the multivariate models, we adjusted for determinants of SAF reported in our previous study, including age, gender, BMI, creatinine clearance, HbA1c, smoking status, packyears, and CVD history [10]. SPSS (version 22, IBM, Armonk, NY, USA) was used for statistical analyses. A p value <0.001 (two-tailed) was considered statistically significant.

Results

Clinical characteristics

Table 1 shows the clinical characteristics of the study population stratified for gender and MetS status. The overall prevalence of MetS was 19% in men and 12% in women.

In both genders, individuals with MetS were older than subjects without MetS and had a significantly higher BMI and waist circumference (all p < 0.001). Both in men and women, the prevalence of MI (3.5% in men and 1.0% in women) and CVA (1.1% in men and 1.2% in women) was higher among individuals with MetS compared to subjects without MetS (p < 0.001). The percentage of current smokers as well as the number of pack-years smoked (p < 0.001) were higher in the participants with MetS. In addition, both men and women with MetS reported higher consumption of coffee compared to individuals without MetS (p < 0.001). Mean SAF levels were significantly higher among both male and female subjects with MetS compared to individuals without MetS (men: 2.07 ± 0.44 AU vs 1.94 ± 0.43 AU, p < 0.001; women: 2.07 ± 0.45 AU vs 1.86 ± 0.42 AU, p < 0.001). In subjects with MetS, mean SAF levels were not different between men and women (2.07 \pm 0.44 AU vs 2.07 \pm 0.45 AU, p = 0.715). However, among individuals without MetS, men had significantly higher SAF levels than women $(1.94 \pm 0.43 \text{ AU vs } 1.86 \pm 0.42 \text{ AU, p} < 0.001)$ (data not shown). Furthermore, mean SAF was 2.33 \pm 0.50 AU in subjects with a history of MI and 1.91 \pm 0.43 AU in subjects without a history of MI (p < 0.001). Mean SAF was 2.22 \pm 0.50 AU in subjects with a history of CVA and 1.91 \pm 0.43 AU in subjects without a history of CVA (p < 0.001) (data not shown). Finally, within obese individuals, subjects with MetS had a higher SAF than individuals without MetS (2.11 \pm 0.47 AU vs 1.94 v 0.41 AU,

Characteristic	Men (n = 32,601)		Women (n = 46,070)	Women (n = 46,070)	
	MetS	Without MetS	MetS	Without MetS	
n (%)	6220 (19)	26,381 (81)	5367 (12)	40,703 (88)	
Age (years)	$49 \pm 11^{*}$	44 ± 13	$50 \pm 12^{*}$	44 ± 12	
Body mass index (kg/m ²)	$29.8 \pm 3.6^{*}$	25.5 ± 3.1	$30.6 \pm 5.1*$	25.1 ± 4.1	
Waist circumference (cm)	$106 \pm 9^{*}$	93 ± 9	$100 \pm 11^{*}$	85 ± 11	
Systolic blood pressure (mmHg)	$141 \pm 14^{*}$	129 ± 14	$138 \pm 16^{*}$	121 ± 15	
Diastolic blood pressure (mmHg)	82 ± 9*	76 ± 9	79 ± 9*	72 ± 9	
Total cholesterol (mmol/L)	$5.4 \pm 1.1^{*}$	5.1 ± 1.0	$5.4 \pm 1.1^{*}$	5.0 ± 1.0	
HDL cholesterol (mmol/L)	$1.04 \pm 0.23^{*}$	1.37 ± 0.30	$1.24 \pm 0.30^{*}$	1.65 ± 0.38	
LDL cholesterol (mmol/L)	$3.56 \pm 0.93^{*}$	3.35 ± 0.89	$3.57 \pm 0.95^{*}$	3.05 ± 0.88	
Triglycerides (mmol/L)	2.03 (1.60-2.73)*	1.04 (0.77-1.40)	1.72 (1.18–2.14)*	0.83 (0.64-1.11)	
Creatinine clearance (mL/min) ^a	$137 \pm 36^{*}$	124 ± 28	$125 \pm 39^{*}$	111 ± 29	
Creatinine clearance (mL/min) ^b	$130 \pm 18^{*}$	128 ± 16	135 ± 21	134 ± 18	
Fasting plasma glucose (mmol/L)	$5.45 \pm 0.55^{*}$	4.98 ± 0.43	$5.33 \pm 0.61*$	4.75 ± 0.42	
HbA1c (%)	$5.7 \pm 0.3^{*}$	5.5 ± 0.3	$5.7 \pm 0.4^{*}$	5.5 ± 0.3	
HbA1c (mmol/mol)	38.5 ± 3.8	36.7 ± 3.2	39.3 ± 3.9	36.5 ± 3.2	
Statin use, n (%)	783 (13)*	1260 (5)	554 (10)*	1029 (3)	
TG-lowering medication, n (%)	44 (1)*	9 (0.01)	16 (0.3)*	3 (0.01)	
BP-lowering medication, n (%)	1445 (23)*	2008 (8)	1919 (36)*	3099 (8)	
Ml, n (%)	214 (3.5)*	289 (1.1)	56 (1.0)*	94 (0.2)	
CVA, n (%)	70 (1.1)*	188 (0.7)	66 (1.2)*	203 (0.5)	
Coffee consumption (cups per day)	4.7 (2.8–5.6)*	3.7 (2.8–5.6)	3.3 (1.9–4.7)*	2.8 (1.3-4.7)	
Smoking status, n (%)					
Non-smokers	2117 (34)*	12,259 (47)	2203 (41)*	19,836 (49)	
Ex-smokers	2425 (39)	8271 (32)	1899 (36)	12,847 (32)	
Current smokers	1629 (27)	5677 (21)	1228 (23)	7698 (19)	
Pack-years					
Ex-smokers	12.8 (6.5–21.9)*	8.2 (3.8–15.0)	7.8 (3.5–15.3)*	5.2 (2.2–10.4)	
Current-smokers	18.0 (10.7–27.0)*	12.9 (6.4–21.5)	16.9 (9.6–26.3)*	11.2 (5.4–18.8)	
SAF (AU)	$2.07 \pm 0.44^{*}$	1.94 ± 0.43	$2.07 \pm 0.45^{*}$	1.86 ± 0.42	
SAF Z-score	$0.21 \pm 0.02^{*}$	0.04 ± 0.01	$0.19 \pm 0.02^{*}$	-0.10 ± 0.01	

Table 1 Clinical characteristics of the study population stratified by MetS status and gender

Data are presented as mean \pm standard deviation, or median (interquartile range) and number (%)

AU arbitrary units, BP blood pressure, CVA cerebrovascular accident, GFR Glomerular Filtration Rate, HDL high density lipoprotein, LDL low density lipoprotein, MetS metabolic syndrome, Mi myocardial infarction, SAF skin autofluorescence, TG triglycerides

* p value < 0.001

^a Creatinine clearance (Cockcroft-Gault formula), ^b Creatinine clearance (CKD EPI)

p<0.001). However, within the population with MetS, there was no significant difference in SAF between obese and non-obese individuals (2.07 \pm 0.45 AU vs 2.06 v 0.45 AU, p=0.46) (data not shown).

The association between SAF and the number of MetS components

In both men and women, we observed a gradual rise in age-adjusted SAF Z-scores with higher number of individuals MetS components. Compared to men without any MetS components, SAF Z-scores were significantly higher among subjects with ≥ 1 MetS component (p < 0.001). Men with ≥ 2 MetS components had significantly higher SAF Z-scores compared to men having only one MetS component (p < 0.001). No significant differences in SAF Z-scores were observed between men with two vs three MetS components as well as men with four vs five MetS components. Among women, SAF Z-scores were significantly higher among those with ≥ 1 MetS component compared to women without any MetS components (p < 0.001). Women with three, four or five MetS components had significantly higher SAF Z-scores

compared to women with only one MetS component (p < 0.001). No differences in SAF Z-scores were found for women with two vs one MetS components as well as three vs two MetS components. Furthermore, women with either four or five MetS components had significantly higher SAF Z-scores than women with two MetS components or three MetS components (p < 0.001). In men, SAF Z-scores tended to be higher in those with one, two and three MetS components while in women SAF

Z-scores were higher in three, four or five MetS components compared to men (Fig. 1).

SAF and the prevalence of the individual MetS components

Figure 2 shows the prevalence of the individual MetS components for both men and women in the total study population according to tertiles of age-adjusted SAF Z-scores. Among men, individuals in the highest SAF





groups had a higher prevalence of elevated blood pressure (58%) compared to subjects in the middle (52%) and lowest SAF group (54%).

We observed the same trend for enlarged waist circumference, impaired fasting glucose, elevated triglycerides and low HDL cholesterol (all p < 0.001).

In contrast to men, the most frequent MetS component in women was enlarged waist circumference (high SAF 48%, intermediate SAF 41% and lowest SAF 41%). There was a clear and significant trend among women within the highest SAF group, to be associated with higher prevalence of blood pressure, elevated triglycerides, lower HDL cholesterol levels and impaired fasting glucose (all p < 0.001).

Logistic regression analysis for the presence of MetS and it components across SAF tertiles

Table 2 shows the results of the logistic regression analyses describing the association between SAF tertiles and the presence of MetS in the total population. Compared to the low SAF group which was set as a reference, high SAF was significantly associated with a higher presence of MetS (odds ratio (OR) 2.61; 95% CI 2.48–2.75). After adjusting for age, gender, BMI, creatinine clearance, HbA1c, smoking status, packyears, CVD history, high SAF (OR 1.24; 95% CI 1.13–1.37) and intermediate SAF (OR 1.11; 95% CI 1.01–1.22) remained significantly associated with the presence of MetS (Table 2, model 3).

Table 2 Unadjusted and adjusted odds ratios for metabolic syndrome and its components associated with tertiles of SAF (total population)

Metabolic syndrome	Model 1		Model 2		Model 3	
	OR	p value	OR	p value	OR	p value
SAF (continuous)	2.37 (2.27-2.48)	<0.0001	1.36 (1.28–1.44)	<0.0001	1.17 (1.08–1.26)	<0.0001
Low SAF	Ref.		Ref.		Ref.	
Intermediate SAF	1.71 (1.62–1.81)	<0.0001	1.13 (1.06-1.21)	<0.0001	1.11 (1.01–1.22)	0.031
High SAF	2.61 (2.48–2.75)	<0.0001	1.38 (1.29–1.48)	<0.0001	1.24 (1.13–1.37)	<0.0001
Elevated blood pressure						
SAF (continuous)	3.03 (2.92-3.14)	<0.0001	1.25 (1.20–1.31)	<0.0001	1.21 (1.14–1.29)	<0.0001
Low SAF	Ref.		Ref.		Ref.	
Intermediate SAF	1.71 (1.65–1.77)	<0.0001	1.06 (1.02-1.11)	0.005	1.04 (0.98–1.11)	0.220
High SAF	2.93 (2.82-3.03)	<0.0001	1.18 (1.12–1.24)	<0.0001	1.16 (1.08–1.24)	<0.0001
Impaired fasting glucose						
SAF (continuous)	2.59 (2.47-2.72)	<0.0001	1.17 (1.10–1.25)	<0.0001	1.09 (1.00-1.19)	0.049
Low SAF	Ref.		Ref.		Ref.	
Intermediate SAF	1.90 (1.78–2.03)	<0.0001	1.09 (1.01-1.17)	0.026	1.08 (0.97-1.20)	0.185
High SAF	3.16 (2.97–3.37)	<0.0001	1.34 (1.24–1.45)	<0.0001	1.27 (1.14–1.42)	<0.0001
Low HDL cholesterol						
SAF (continuous)	1.08 (1.03-1.12)	0.001	1.46 (1.39–1.54)	<0.0001	1.27 (1.19–1.36)	<0.0001
Low SAF	Ref.		Ref.		Ref.	
Intermediate SAF	0.96 (0.92-1.01)	0.082	1.08 (1.02–1.13)	0.004	1.02 (0.94–1.10)	0.663
High SAF	1.01 (0.96–1.05)	0.786	1.27 (1.20–1.34)	<0.0001	1.13 (1.04–1.22)	0.003
Elevated triglycerides						
SAF (continuous)	1.78 (1.70–1.85)	<0.0001	1.29 (1.22–1.37)	<0.0001	1.08 (1.00–1.16)	0.029
Low SAF	Ref.		Ref.		Ref.	
Intermediate SAF	1.49 (1.41–1.57)	<0.0001	1.12 (1.05–1.18)	<0.0001	1.04 (0.95–1.13)	0.426
High SAF	1.89 (1.80–1.98)	<0.0001	1.34 (1.25–1.42)	<0.0001	1.17 (1.07–1.28)	0.001
Enlarged waist circumferen	ce					
SAF (continuous)	1.89 (1.82–1.95)	<0.0001	1.31 (1.23–1.39)	<0.0001	1.32 (1.21–1.44)	<0.0001
Low SAF	Ref.		Ref.		Ref.	
Intermediate SAF	1.45 (1.40–1.50)	<0.0001	1.11 (1.05–1.18)	0.001	1.14 (1.04–1.25)	0.004
High SAF	1.93 (1.86–2.00)	<0.0001	1.26 (1.18–1.35)	<0.0001	1.32 (1.20–1.46)	<0.0001

Data are presented as odds ratios (95% confidence interval) per arbitrary unit (AU). SAF groups were based on tertiles of age-adjusted SAF Z-scores. Model 1 = unadjusted; model 2 = adjusted for age, gender, BMI; model 3 = adjusted for age, gender, BMI, HbA1c, creatinine clearance, current smoking, pack-years, MI and CVA

Significant associations are shown in italic

BMI body mass index, CVA cerebrovascular accident, HDL high density lipoprotein, MI myocardial infarction, OR odds ratio, SAF skin autofluorescence

Considering the individual MetS components, high SAF (OR 3.16; 95% CI 2.97–3.37 and intermediate SAF (OR 1.90; 95% CI 1.78–2.03) were significantly associated with the presence of impaired fasting glucose, although the OR attenuated after adjusting for several covariables (Table 2, models 1, 2 and 3). We also found high SAF (OR 2.93; 95% CI 2.82–3.03) and intermediate SAF (OR 1.71; 95% CI 1.65–1.77) to be associated with the presence of elevated blood pressure (Table 2, model 1). Finally, high SAF (OR 1.45; 95% CI 1.86–2.00) and intermediate SAF (OR 1.45; 95% CI 1.40–1.50) were also associated with the presence of enlarged waist circumference, which remained significant after adjusting for covariables (Table 2, models 1, 2 and 3).

Associations for SAF in the metabolic syndrome population

The univariate associations between the individual MetS components and SAF levels in the MetS population are shown in Table 3. In subjects with MetS, the impaired

Table 3 Associations for skin autofluorescence in the metabolic syndrome population

	Coefficient B	SE	p value
Univariate model			
Elevated blood pressure	0.113	0.012	<0.0001
Low HDL cholesterol	-0.094	0.009	< 0.001
Elevated triglycerides	0.029	0.009	0.001
Impaired fasting glucose	0.124	0.008	<0.0001
Enlarged waist circumference	0.038	0.011	0.001
Multivariate model			
Elevated blood pressure	0.044	0.015	0.003
Low HDL cholesterol	0.036	0.012	0.002
Elevated triglycerides	-0.001	0.011	0.920
Impaired fasting glucose	0.008	0.012	0.502
Enlarged waist circumference	0.016	0.014	0.269
Age	0.019	0.001	1.3×10^{-159}
Male gender	-0.016	0.011	0.272
Creatinine clearance (mL/min)	0.001	0.0002	0.014
HbA1c (%)	0.055	0.016	2.3×10^{-4}
Coffee consumption (cups/ day)	0.024	0.002	6.6 × 10 ⁻²⁹
Current smoking ^a	0.116	0.011	1.1×10^{-23}
Pack-years	0.004	0.0003	5.6×10^{-31}
MI	0.062	0.029	0.033
CVA	0.026	0.043	0.562

Data are shown as coefficient B (standard error) per arbitrary unit (AU). The individual MetS components were included into the model as categorical variables (yes/no) while the other variables were continuous variables

Significant associations are shown in italic

CVA cerebrovascular accident, HDL high density lipoprotein, MI myocardial infarction

^a Current smoking vs non- and former smoking

fasting glucose and elevated blood pressure components gave the strongest increase in SAF. Subjects with MetS having the impaired fasting glucose component had a 0.12 AU higher SAF compared to subjects without this particular component. A similar association was observed for individuals with MetS having the elevated blood pressure who had a 0.11 AU higher SAF than those without elevated blood pressure. Multivariate analyses showed that after adjusting for several determinants of SAF, elevated blood pressure (0.05 AU) and low HDL cholesterol (0.04 AU) were significantly associated with higher SAF. Impaired fasting glucose, elevated triglycerides and enlarged waist circumference were not significantly associated with SAF. Regarding the clinical and lifestyle factors, age, HbA1c, current smoking, packyears and a history of CVD were all significantly associated with higher SAF whereas a higher creatinine clearance was associated with lower SAF.

Discussion

In the present study, we have demonstrated that SAF levels were higher in both male and female participants with MetS compared to those without MetS. Furthermore, SAF was significantly and independently associated with the presence of MetS and some of its individual components, particularly elevated blood pressure, impaired fasting glucose and enlarged waist circumference.

One of the main findings of the present study was that we observed significantly higher SAF levels in subjects with MetS compared to individuals without MetS, which is in line with a study by Den Engelsen et al. [33]. These authors observed higher SAF levels in obese subjects with MetS compared to those without MetS. The latter group may be considered as "healthy obese", which is also reflected by their SAF level. Additional analyses on our data showed a similar pattern (data not shown). In our study, 45% of men and 51% of women with MetS were obese, as defined by a BMI above 30 kg/m². However, within the population with MetS, we did not observe a statistically significant difference in SAF between obese and non-obese individuals. Within the same population, subjects with an enlarged waist circumference had higher SAF than subjects without an enlarged waist circumference. These findings imply that not general obesity but an enlarged waist circumference, or visceral obesity has more impact on SAF within individuals with MetS.

Next, our results may not be compared directly to those from Monami et al. [34] who have also shown that SAF levels are elevated among diabetic individuals with MetS. Mean SAF was higher than in our study as their cohort primarily consisted of type 2 diabetic individuals with a mean diabetes duration of 12 years. Individuals with type 2 diabetes have in general higher SAF than subjects without diabetes [9]. Furthermore, subjects in their study were older and had a higher prevalence of CVD, both are associated with higher SAF [11, 14].

In addition to the observation of higher SAF levels in subjects with MetS, we also found that a higher number of individual MetS components coincides with even higher SAF Z-scores. A previous study has already shown that a higher number of MetS components was associated with higher serum AGEs levels [35]. Clinical studies have demonstrated that a higher number of MetS components is associated with both incident CVD and type 2 diabetes [36-38]. Klein et al. showed that individuals with one MetS components had 2.5% risk of incident CVD in the next 5 years whereas subjects with four or more MetS components had an almost 15% risk. The 5-years risk for type 2 diabetes increased from 1.1% (one MetS component) to 17.9% (\geq four MetS components) [36]. An 11-year follow-up study demonstrated an almost linear relationship between the number of individual MetS components and the risk of coronary heart disease in subjects without a history of CVD or type 2 diabetes [38]. Therefore, we suggest that an increase in SAF may reflect an even higher risk of type 2 diabetes and CVD.

It has been reported that the prevalence of both MetS and its components differ between men and women [39]. In men, we observed that elevated blood pressure was the most prevalent MetS component. Moreover, we found that subjects in the highest SAF group had a higher prevalence of elevated blood pressure compared to individuals in the intermediate or lowest SAF group. A few studies have described the association between SAF and elevated blood pressure, but the results are contradictory. For example, in renal transplant recipients, systolic blood pressure was positively associated with SAF [40] whereas neither systolic nor diastolic blood pressure was related to SAF in a recent Japanese study among type 2 diabetic individuals [41]. A recent study by Botros et al. [42] showed that both systolic and diastolic blood pressure were significantly associated with SAF. They showed that individuals with elevated blood pressure had a higher odds for having a SAF level > median, compared to subjects without elevated blood pressure. Elevated blood pressure may well be a consequence of increased AGE accumulation. A recent study demonstrated that carotidfemoral pulse-wave velocity and central pulse pressure were independently associated with plasma AGEs [43]. Several AGEs are able to form crosslinks within collagen in the vascular wall, which may result in impaired vascular elasticity and increased arterial stiffness, causing blood pressure to rise [44, 45]. Our data together with previous studies suggest that AGE accumulation may indeed be involved in the underlying pathophysiology of elevated blood pressure.

In contrast to men among we found elevated blood pressure to be the most prevalent component, we observed that enlarged waist circumference was the most prevalent MetS component among women. Individuals with high SAF levels had a higher presence of enlarged waist circumference compared to subjects with intermediate or low SAF levels. However, in the multivariable analyses, enlarged waist circumference was not significantly associated with SAF. The correlation between waist circumference or BMI and higher SAF levels has been demonstrated among subjects with type 2 diabetes [34]. Recently, it has been shown that SAF levels were elevated among individuals with central obesity compared to lean subjects [33]. Interestingly, Angoorani et al. [46] have demonstrated that dietary consumption of AGEs is associated with MetS, and in particular abdominal obesity. Furthermore, it was observed that an increased consumption of food AGEs was associated with an even higher risk of (abdominal) obesity. They reported that individuals with a high AGEs consumption had a higher fat intake, and indeed it is known that fat contains a significant amount of dietary AGEs per gram of weight [47]. Another possible mechanism that has been reported to increase AGEs accumulation in obese individuals is oxidative stress [48, 49]. Autoxidation of lipoproteins result in the formation of advanced lipoxidation end products formation (ALEs) such as carboxymethyllysine (CML) [1].

Next, we have shown that higher SAF is significantly associated with an increased presence of impaired fasting glucose. After adjusting for HbA1c, impaired fasting glucose was not associated with SAF probably as a consequence of high collinearity. A previous study already demonstrated that among subjects with central obesity, fasting glucose levels were significantly associated with higher SAF in univariate regression analyses but not after adjusting for covariables [33]. Another recent study reported no significant difference in plasma AGEs between women with normal fasting glucose and impaired fasting glucose [50]. As we included in our study subjects without diabetes only, and glucose levels were relatively low (<6.9 mmol/L), this may be the reason why fasting glucose was not associated with SAF. In individuals with diabetes, the formation of AGEs is accelerated particularly due to (chronic) hyperglycaemia [51]. Glucose plays an essential role in the formation of AGEs as protein amino groups and lipids are non-enzymatically glycated to form stable structures on long-lived tissues [4, 52]. A second pathway which leads to the formation of AGEs is through autoxidation of glucose by reactive oxygen species and through formation of carbonyl compounds [1]. It has been shown that AGEs are involved in beta-cell injury, probably caused by inflammation and oxidative stress thought the AGE–RAGE interaction [53]. This may be prevented by consumption of a low-AGE diet, which has been suggested to improve insulin sensitivity [54, 55].

After adjusting for several determinants, having a low HDL cholesterol was significantly associated with higher SAF levels but elevated triglycerides was not. Among individuals with MetS, HDL was just above the limit in men and slightly decreased in women, while statin use was 13% in men and 10% in women. Additional analysis showed that statin users had significantly higher SAF Z scores than those not using statins, also after correcting for age. Triglycerides were slightly elevated in women, but higher in men. Around 1% of individuals with MetS used triglycerides lowering medication. There was no significant difference in SAF Z scores between subject using triglycerides lowering medication and subjects not using this kind of medication. Limited data exist on the association between SAF and both HDL cholesterol and triglycerides. In subjects with type 2 diabetes, HDL cholesterol levels were negatively, and triglycerides were positively associated with SAF [14, 34]. Similar associations have been reported between serum AGEs levels and triglycerides and HDL cholesterol levels in diabetes [56]. In addition, higher consumption of AGE-rich foods was associated with hypertriglyceridemia [46]. HDL cholesterol has been reported to inhibit oxidative modification of LDL cholesterol [57]. A study in 200 type 2 diabetic subjects showed that higher anti-oxidative capacity of HDL was associated with lower SAF but not plasma HDL cholesterol levels itself [58]. Circulating AGEs may impair the capacity of HDL to protect against oxidation of LDL cholesterol which potentially increases oxidative stress. In turn this might accelerate the formation of AGEs [59] and plays a role in atherosclerosis [60].

The strength of this study is its large and well-characterized study population, including high quality data on anthropometric and clinical measurements. This resulted in a good statistical power and the ability to perform stratified analysis. Moreover, this is the largest study in subjects without diabetes and impaired renal failure showing the association between SAF and several cardiometabolic risk factors. A limitation of the study includes the cross-sectional design, which does not allow us to draw any conclusions about causality in the association between SAF levels and the risk of cardiometabolic diseases.

Conclusion

The present findings of elevated SAF levels in subjects with MetS, the positive association between the number of individual MetS components and higher SAF levels, as well as the observation that higher SAF levels are associated with higher prevalence of the individual components, provide further evidence that accumulation of AGEs may contribute to the pathophysiology of several cardiometabolic risk factors. Prospective studies are needed to demonstrate whether SAF measurement can be used as an additional non-invasive screening tool to detect individuals at high-risk for both CVD and incident type 2 diabetes.

Abbreviations

AGEs: advanced glycation end products; AU: arbitrary units; BMI: body mass index; CVD: cardiovascular disease; HDL: high density lipoprotein; MetS: metabolic syndrome; SAF: skin autofluorescence.

Authors' contributions

BHRW was the primary investigator. RPW and BHRW contributed to the study design. RPW performed the statistical analyses. RPW, BHRW, MMvdK, HLL, JVO, RG, SNS and ADP contributed to interpretation of the data and analyses. RPW drafted the manuscript. All authors read and approved the final manuscript.

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Competing interests

RG is founder and shareholder of DiagnOptics BV, Groningen, the Netherlands, manufacturer of the AGE Reader (http://www.diagnoptics.com) which has been used in the present study. All authors declare that they have no competing interest.

Availability of data and materials

The manuscript is based on data from the LifeLines Cohort Study. LifeLines adheres to standards for data availability. The data catalogue of LifeLines is publicly accessible on http://www.lifelines.net. All international researchers can apply for data at the LifeLines research office (LLscience@umcg.nl). The LifeLines system allows access for reproducibility of the study results.

Ethics approval and consent to participate

The study was approved by the Medical Ethical Committee of the University Medical Center Groningen. Informed consent was obtained from all individual participants included in the Lifelines study.

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Therapeutic interventions against accumulation of Advanced Glycation End products (AGEs)

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Abstract

Advanced Glycation End products (AGEs) are formed in a non-enzymatic reaction between reducing sugars and proteins, lipids or nucleic acids. AGEs build up in the body naturally during aging and are involved in the development of several pathologies such as diabetic complications, atherosclerosis and cardiovascular disease. Since AGE levels are a good predictor of diabetic complications and cardiovascular mortality, AGE measurements can provide new information to the current prognosis and treatment options of diabetic patients. Moreover, research regarding interventions to reduce AGE accumulation has been of major interest the latest years. This review examines interventions that have been studied in clinical trials or in in vivo studies when that compound is currently available for human use as well. Interventions can be aimed at different levels in the AGE formation pathway and depend on different mechanisms, among which antioxidant ability, scavenging of reactive carbonyl species (RCS) or breaking AGE-induced crosslinks. Pharmaceutical options show promising results, yet their clinical relevance is doubtful so far due to safety concerns. For individuals with high AGE levels but no clinical symptoms, lifestyle interventions such as a low AGE diet and physical exercise might be more effective. Nutraceuticals, derived from food sources and available as dietary supplements, have mostly been investigated in pre-clinical studies and showed positive effects on diabetic complications such as nephro- and retinopathy. Due to the deleterious effects of AGEs on diabetes and its complications, AGE-inhibitors are interesting agents to investigate more extensively.

Keywords

advanced glycation end products (AGEs), diabetic complications, therapy, lifestyle interventions

Introduction on Advanced Glycation End products

Advanced glycation end products (AGEs) are a diverse set of compounds that accumulate in tissues during normal ageing and contribute to a range of diseases such as diabetes mellitus and its complications, neurodegeneration and inflammation.¹ AGEs are generated when sugars react with proteins, lipids or nucleic acids in a non-enzymatic way. This glycation process is described as the Maillard reaction and is known for the browning of foods. This reaction is characterised by a few steps with intermediate products to eventually form AGEs. When the carbonyl group of reducing sugars react with the aminoterminal group of proteins, an unstable Schiff base is formed in a reversible process. During rearrangements, the more stable Amadori product is produced, e.g. the glycated haemoglobin HbA1c. When further reactions as rearrangements, oxidation and dehydration take place, AGEs will be produced.² During these rearrangements highly reactive intermediate α-dicarbonyls, also known as reactive carbonyl species (RCS), accumulate and cause carbonyl stress. Examples of these products are 3-deoxyglucosone (3-DG) and methylglyoxal (MGO).³ RCS and AGE formation can also occur by glycoxidation or lipid peroxidation.4

Under physiological circumstances, the endogenous AGE production will take weeks or years and long-lived proteins such as collagen are the major target. Under stress conditions such as hyperglycemia or oxidative stress this reaction accelerates and can also affect short-lived substrates (e.g. enzymes and

hormones), inducing structural changes.⁵

AGEs can also come from exogenous sources such as food of which 10% is estimated to be absorbed in the gut.⁶ Animalderived foods, high in fat and protein, consist of high AGE levels. In contrast, food that is enriched with carbohydrates, such as whole grains, vegetables and fruit, are generally low in AGEs. Since high temperatures can accelerate AGE formation, food processing by heating can contribute to the accumulation of AGEs in the body.⁷ Furthermore, smoking is an important exogenous source of AGEs.⁸

Role of AGEs in health and disease

The formation of AGEs and accumulation in the body are natural processes during ageing. Aging is explained as a multifactorial process leading to a gradual decline in physiological functions, affecting all tissues in the body.⁹ High rates of AGE accumulation in the skin have been shown to correlate with aging¹⁰ and excessive AGE accumulation can accelerate the aging process. The amount of AGEs is based on the rate of formation, determined by ROS and reducing sugars, and the rate of clearance, determined by the activity of the glyoxalase system, where glyoxalase I (Glo I) is able to detoxify reactive carbonyl compounds.¹¹ Aging can cause an imbalance in this system, since ROS is present in a larger extent while Glo I activity is decreased.¹ Furthermore, AGE accumulation is aggravated in some chronic diseases as well, such as cardiovascular disease, diabetes mellitus, renal failure and Alzheimers disease.² AGEs



Fig 1. During the traditional Maillard reaction reducing sugars react with the amino group of proteins to form intermediate products Schiff bases and Amadori products and eventually AGEs. More recently it appeared AGEs can be formed through other pathways such as glycoxidation and lipid peroxidation where reactive carbonyl species (RCS) are generated that covalently bind to proteins. (CML- carboxy-methyl-lysine; CEL- carboxy-ethyl-lysine; GO: glyoxal; MGO- methylglyoxal; 3-DG- 3-deoxyglucosone)

can damage cells and tissues through several mechanisms and thereby contribute to aging or disease.

First, AGEs can bind to certain receptors (RAGE; receptor for Advanced Glycation End products) on different cells. This induces several signalling cascades, among which activation of MAP kinases and the JAK/STAT pathway.^{14,15} Many of these signalling pathways lead to the activation of transcription factors such as NF κ B, which induces a diverse set of target genes. Pro-inflammatory genes (e.g. TNF- α , IL-1 and IL-6), adhesion molecules (e.g. VCAM-1) and vasoconstrictors are activated.^{1,14} In addition, reactive oxygen species (ROS) are generated by activation of NADPH oxidases and then stimulate the further formation of AGEs.¹⁵ Oxidative stress and inflammation can in their turn elicit tissue damage and lead to accelerated aging.⁹

Besides a receptor-mediated response, AGEs are responsible for alterations in protein function. Glycation of (intracellular) proteins can alter their structure and lead to impaired function of growth factors, enzymes and transcription factors, contributing to impaired cell function.⁵ Furthermore, AGEs stimulate the formation of crosslinks between (intracellular) proteins, and can trap (lipo)proteins.¹² Accumulation of AGEs in the extracellular matrix (ECM) can result in crosslinking of collagen molecules leading to stiffness and decreased elasticity of tissues.¹⁶ Particularly tissues rich in ECM and longlived proteins such as skin, skeletal muscles, tendons, heart and lens are targeted by this stiffening and is associated with aging.¹⁷ Under pathological conditions the consequences of crosslinking by AGEs include thickening of the capillary basement membrane, rigid vessels and development of atherosclerosis and glomerular sclerosis.¹⁸

In patients suffering from diabetes or renal disease, AGEs accumulate more rapidly due to hyperglycaemia, oxidative stress or impaired renal clearance. AGEs then contribute to the progression of these diseases and complications such as diabetic neuropathy, nephropathy and the formation of cataract. Atherosclerosis is the major cause of death in diabetic patients¹⁹ and is characterized by cross-linking of extracellular matrix proteins in the vessel wall by AGE accumulation, thereby trapping plasma proteins.²⁰ Moreover, AGEs in the vessel wall interfere with the nitric oxide (NO)-mediated relaxation ability of the endothelium.²¹

NF-κB signalling and ROS induce apoptosis of pericytes and endothelial cells,²² contributing to diabetic retinopathy. This is enhanced by hyperpermeability of capillaries, resulting in vascular leakage.²³ In addition, the thickening of the capillary basement membranes by increased synthesis of collagen and other matrix molecules is a mechanism by which retinopathy is strengthened. ²⁴ The same mechanisms play a role in the pathophysiology of diabetic nephropathy. Apoptosis of mesangial cells²⁵ and the thickening of the glomerular basement membrane is partly responsible for altered filtration, albuminuria and eventually renal failure.²

AGE accumulation represents 'glycaemic memory': the phenomenon that explains the sustained beneficial effects long after a period of intensive glycaemic control, as well as the prolonged harmful effects after hyperglycaemia.^{26,27} Together with their role in diabetic complications, measuring

AGEs is emerging as a tool to predict the odds of developing complications and detect patients at risk. The AGE Reader (Diagnoptics, Groningen, the Netherlands) provides a non-invasive, quick and reproducible way for the AGE-related skin autofluorescence (SAF). Skin AGE levels proved to be an independent predictor of microvascular complications in type 2 diabetes.²⁸ Furthermore, skin autofluorescence is, except for age, the best predictor for (cardiovascular) mortality and provides additional information to conventional CV risk assessment engines.²⁹



diabetic complications

Fig. 2 AGEs contribute to aging and disease by different mechanisms. Glycation of proteins alters their structure and function, leading to impaired cell function. AGE-RAGE interaction activates NFkB, inducing several cellular responses, such as apoptosis, production of pro-inflammatory cytokines and ROS. Finally, AGEs form crosslinks between collagen and other proteins, leading to tissue stiffening. These are key processes in many diabetic complications, such as atherosclerosis, diabetic nephropathy and diabetic neuropathy.

Interventions to reduce AGEs

Since AGEs have shown to play an important role in aging and the development and progression of many chronic diseases, they are an excellent target for new therapies. To diminish the harmful effect of AGEs on cellular and tissue functioning, interventions are proposed that either avoid the further formation or accumulation of AGEs or remove the AGEs that are already formed. This review gives an overview of existing and potential interventions that can inhibit AGEs now or in the near future, which are classified in pharmacologic, lifestyle and nutraceutical interventions.

Pharmacologic interventions

Researchers have been ambitious to find substances with AGE-reducing properties. Compounds that have been studied extensively are Aminoguanidine (AG) and Alagebrium (ALT-

711).

Aminoguanidine (AG) is a small molecule that reacts with dicarbonyl compounds (e.g. MG and 3-DG) and Amadori intermediates to inhibit the formation of AGEs.³⁰ The first clinical trial (ACTION) was performed to evaluate the effect of AG on the further development of diabetic nephropathy. A large cohort of 690 patients with T1DM and known nephro- and retinopathy participated and were treated for 2-4 years with AG. Overall, a significant reduction of diabetes complications was observed. AG administration reduced the 24-hour proteinuria and could prevent the decrease in glomerular filtration rate. However, the inhibition of AGE formation by AG showed no effect on serum creatinine.³¹ A second trial, involving AG therapy in T2DM patients, was early terminated due to undesirable side effects such as abnormalities in liver

function, gastrointestinal problems and anemia.³²

Alagebrium (ALT-711) has the ability to break crosslinks of Maillard reaction products and demonstrated positive effects on atherosclerosis and diabetic nephropathy in vivo. Clinical studies that have been performed mostly investigated the use of Alagebrium in patients with hypertension or heart failure. Two studies that examined hypertensive patients treated with Alagebrium for a relative short period (8-10 weeks)^{33,34,} showed some beneficial effects on several cardiovascular variables, such as an increase in arterial compliance and a decrease of arterial pulse pressure.³³ Furthermore endothelial function was improved and the therapy might reduce arterial remodelling. Nevertheless, the same study reported no changes in cardiac output, blood pressure and systolic or diastolic function and other cardiovascular variables.³⁴ In a study where 23 patients with diastolic heart failure were treated for four months ambiguous results were found. Although diastolic function and left ventricular mass were improved, no change in VO2 max, blood pressure or aortic distensibility were found.³⁵ Another study observing 102 heart failure patients after 9 months of treatment did not show positive results and could not find an improvement of diastolic and systolic function, AGE accumulation or New York Heart Association (NYHA) classification.³⁶ Similarly, no convincing results on hemodynamics or exercise capacity could be detected after 1 year of treatment in healthy individuals.^{37,38}

Due to the mentioned safety and efficacy problems, it is implausible that Aminoguanidine and Alagebrium will be used for the treatment or prevention of diabetic complications.

Azeliragon is a RAGE inhibitor and has been tested in clinical trials to diminish Alzheimers Disease (AD). RAGE is not only a receptor for AGEs, but can bind amyloid β as well. In AD, RAGE expression is upregulated in the brain and contributes to inflammation, oxidative stress and neurodegeneration.³⁹ RAGE antagonist Azeliragon (also known as TTP488 or PF-04494700) was administered to 399 patients for 18 months to inhibit the interaction between RAGE and amyloid β and block signal transduction.⁴⁰ This also might be interesting to counteract the detrimental effects of AGEs through its receptor. A low dose was suggested to have a decreased decline on the Alzheimer's Disease Assessment Scale-cognitive (ADAS-cog), a test that determines parameters as memory, reasoning, language and orientation.⁴¹ Nevertheless, there was no significant difference in other clinical markers and the study was terminated early.⁴⁰ The drug was developed earlier for diabetic neuropathy but this study was discontinued as well.

Besides pharmaceuticals that specifically target AGEs, there is another subset of generic drugs, initially developed to decrease blood pressure or cholesterol, but happen to have an effect on AGEs as well. Several small studies on metformin, glucose-lowering medication, showed some beneficial effects on glycation measures, such as a decrease of MG⁴² and lower AGEs and oxidative stress in patients with type 2 diabetes.⁴³ Not all studies were able to find additional effects, therefore the AGE-inhibition capacity of metformin might be attributed merely to improved glycaemic control instead of dicarbonyl quenching.

Lipid-lowering medication might inhibit AGE formation as well due to anti-oxidative properties, which partly reduces

lipid peroxidation. Atorvastatin showed a decrease in serum AGEs in non-alcoholic steatohepatitis (NASH) patients with dyslipidemia after 12 months of treatment.⁴⁴ Serum AGEs were also reduced in patients with non-diabetic chronic kidney disease and dyslipidemia after one year of atorvastatin treatment.⁴⁵ This effect was also observed in diabetic patients that received cerivastatin for three months.⁴⁶ In addition to the decrease in serum AGEs, simvastatin showed a decrease of RAGE expression in carotid artery plaques, by inhibition of AGE formation.⁴⁷

Finally, blood pressure-regulating medication also has a potential effect on AGEs. Until now, only one small study on ACE-inhibitors has evaluated the effect on AGE formation. Ramipril treatment for two months decreased fluorescent AGEs, but not non-fluorescent CML, alongside reduced blood pressure and proteinuria.⁴⁸

Furthermore, Angiotensin Receptor blockers have shown in several small studies that they possess AGE-reducing effects. One year of valsartan treatment decreased serum AGEs, but did not affect other metabolic and oxidative markers in diabetic hypertensive patients.⁴⁹ Candesartan administration to diabetic patients for three months decreased urinary AGEs⁵⁰ and in addition slightly improved creatinine clearance in diabetic kidney disease patients.⁵¹ In contrast, a larger randomised controlled trial with a longer follow-up period could not detect a treatment effect of irbestartan on AGEs in type 2 diabetic subjects with microalbuminuria.⁵²

Lifestyle interventions

Pharmaceutic interventions against AGEs are not approved yet to be clinically used. For individuals that have high AGE levels but no clinical signs of disease yet, non-medical interventions such as adopting a healthy lifestyle might be a more effective approach to prevent further AGE accumulation and increase healthspan (i.e. the disease-free time of life).

Low AGE diet

Since the composition and especially the preparation of food largely determine the amount of exogenous AGE intake, a diet low in AGEs can reduce the absorbed AGEs from the gut. Several studies on a low AGE diet have been completed, using different study populations (healthy and obese subjects as well as patients with diabetes and renal failure). The duration of the low AGE differed between studies, but were all between 1 and 4 months. The decrease of AGEs in the diet was between 30 and 60%, which was generally due to differences in cooking methods. In all studies an isocaloric, low AGE diet showed a decrease in serum AGEs and in most studies, except for one⁵³, this decrease is accompanied by a reduction in markers of inflammation and oxidative stress.54-59 In diabetic and obese subjects with insulin resistance, the HOMA-determined insulin sensitivity improved.^{60,61} A calorierestricted diet reduced plasma AGEs as well, which can be due to a reduced intake of food AGEs or because of other mechanisms such as upregulation of sRAGE or decreased ROS formation.⁶² It must be noted that AGE intake can largely differ in different populations and countries due to differences in the preparation of food. The effects of a low AGE diet should not be undermined since the contribution of dietary AGEs is larger than the endogenously amount of formed AGEs in plasma.63

Physical exercise

Physical exercise has shown to be protective against cardiovascular disease, increases longevity and is an important tool to prevent the development of diabetes in subjects with impaired glucose tolerance.⁶⁴. The influence of exercise on AGE levels has been described in several studies.

The first study investigated the effect of short and long runs on changes in methylglyoxal (MG) content in red blood cells of trained and untrained students. Long runs showed to have the largest reduction in MG concentration; 41% and 60% in untrained and trained students respectively. ⁶⁵ Another study explored the influence of life-long endurance running on the accumulation of AGEs in connective tissue and found that life-long runners had a 21% lower AGE crosslink density of pentosidine in patellar tendons, accompanied by an 11% decrease in skin AGE levels.⁶⁶ Doing Tai Chi, an exercise of moderate intensity and an aerobic nature, for twelve months, showed a decrease in serum AGEs, most likely by stimulating antioxidant enzymes that reduce oxidative stress.⁶⁷ In addition, a study involving middle-aged females in a 12-week lifestyle modification demonstrated a decrease in serum AGEs, as well as reductions in body fat and serum HDL-cholesterol compared to the control group.⁶⁸

Furthermore, in a 6 months interventional program that was focused on stimulating mild to moderate physical activity in Japanese elderly, a reduction in serum sRAGE was demonstrated. The decrease in sRAGE levels could be explained by a reduction of plasma AGEs, which in its turn can inactivate RAGE expression as well as sRAGE circulation as a scavenger of AGEs.⁶⁹ In contrast, moderate exercise for six months in type 2 diabetic women resulted in increased sRAGE levels and improved cardio-metabolic risk factors. This possibly improves scavenging of AGEs by sRAGE and decreases activation of the AGE-RAGE pathway, preventing cellular dysfunction.⁷⁰ Since these studies show opposing effects, which might be due to the study population, the clinical relevance of the relationship between exercise and sRAGE should be studied further.

Finally, in a 12-week study obese men participated in either a low AGE diet, physical (aerobic) exercise (45 minutes with an intensity of 65-75% of maximum heart rate, three times a week) or a combination of both. In contrast to the other studies, an AGE-reducing effect of performing exercise alone was not perceived. Only in combination with the low AGE diet, this intervention provided a decrease in serum CML and MG.⁷¹

Nutraceuticals

Instead of synthetic pharmaceuticals, compounds from natural sources have attracted attention to inhibit AGEs. Natural AGE inhibitors can be found in vegetables, fruit, tea and medicinal plants and many of them are available as dietary supplements.

Vitamin B and derivatives

It has been reported that diabetic patients have a substantial deficiency of vitamin B1.⁷² Vitamin B6 (pyridoxamine and pyridoxine) and vitamin B1 (thiamine and the synthetic prodrug benfotiamine) supplementation have been described as a potent AGE inhibitory strategy.

Pyridoxamine can inhibit the conversion of Amadori products to AGEs and is able to scavenge reactive oxygen species and

the reactive carbonyl intermediates that are products of sugar and lipid degradation.⁷³ Although in vivo experiments demonstrated the efficient inhibition of AGEs by pyridoxamine along with positive effects on diabetic nephropathy,⁷⁴ clinical evidence is more ambiguous. In 2007, a phase 2 trial including patients with kidney disease due to type 1 or 2 diabetes, showed the inhibiting effect of pyridoxamine treatment (Pyridorin; NephroGenex, Inc.) on plasma AGEs. Additionally, 6 month pyridoxamine treatment suggested the potential to slow down renal disease by a reduced change in serum creatinine from baseline and urinary TGF β excretion.⁷⁵ In contrast, a second trial in patients with type 2 diabetic nephropathy could not repeat this effect on creatinine, although patients with less renal impairment might profit. It is suggested that the effect of AGE inhibition is more effective in an earlier stage, which may be before the onset of pathologic changes.⁷⁶

The effect of high-dose thiamine (vitamin B1) therapy was examined in a pilot study with type 2 diabetic patients with microalbuminuria, and reported decreased urinary albumin excretion after 3 months but no effect on dyslipidaemia, glycaemic control or blood pressure.77 Benfotiamine is a prodrug of thiamine with a higher bioavailability, and in vitro data indicates that it inhibits three major pathways that elicit hyperglycaemic vascular damage, among which the AGE formation pathway. Benfotiamine is able to inhibit these pathways simultaneously by increasing the activity of the transketolase enzyme. In addition, NF-κB activation could be prevented by benfotiamine. These effects were able to reduce diabetic retinopathy in an diabetic animal model.⁷⁸ Human studies on benfotiamine are still inconclusive. Studies showing positive results include a beneficial effect on endothelial function, oxidative stress and AGE levels after consumption of a high AGE meal.⁷⁹ Another study reported normalisation of several indicators of hyperglycaemia including AGE formation, although this last study only showed positive effects in combination with alpha-lipoic acid.⁸⁰ Alternatively, Alkhalaf et al. concluded in two studies that benfotiamine treatment for 12 weeks did neither reduce urinary albumin excretion (UAE) and excretion of a tubular damage marker, nor did it affect plasma or urinary AGEs and plasma markers of endothelial dysfunction.^{81,82}

Benfotiamine might be profitable for patients with diabetic polyneuropathy. In a double blind, placebo-controlled phase 3 trial, benfotiamine treatment for six weeks improved the Neuropathy Symptom Score (NSS) in the per protocol analysis, with the greatest improvement in the parameter pain.⁸³ Other studies examined the effect of benfotiamine in combination with other B vitamins. A combination of benfotiamine with vitamin B6 and B12 for 12 weeks improved the nerve conduction velocity in the peroneal nerve, and this result was repeated in a 9 month intervention study in 9 patients.⁸⁴ A different study on Milgamma-N (benfotiamine-vitamin B combination) reported therapeutic effects after six weeks on parameters pain and vibration sensation.⁸⁵ However, these results were contradicted in a 24-month study examining the effect of benfotiamine (Benfogamma, Wörwag Pharma) in type 1 diabetic patients without clinical neuropathy. No beneficial effects on peripheral nerve function or inflammatory biomarkers were reported, which might be explained by differences in study population, where an improvement in patients with almost normal nerve function might be unfeasible.⁸⁶



Fig. 3 The deleterious effects of AGEs can be stopped by reducing AGE accumulation and decrease RAGE signalling. There are several aspects in the AGE formation pathway that can be targeted by pharmaceutic, lifestyle and nutraceutical interventions.

Carnosine

L-carnosine is a naturally occurring dipeptide that is primarily present in the central nervous system and skeletal muscles. In vitro studies demonstrated an effective anti-glycosylating effect by reacting with RCS and inhibiting protein crosslinking.⁸⁷ Other protective functions of carnosine are its antioxidant activity by scavenging of reactive oxygen species.⁸⁸

The results obtained from in vitro studies make carnosine an interesting potential therapeutic agent. In contrast to rodents, humans possess the carnosinase enzyme, which can degrade carnosine through hydrolysis. Therefore, carnosinase-resistant derivatives such as D-carnosine and its more bioavailable prodrug D-carnosine-octylester (DCO) have been developed. D-carnosine has been proved to have the same efficiency as L-carnosine in quenching RCS and diminishes the development of renal disease and dyslipidaemia in obese Zucker rats.⁸⁹ Moreover, treatment with DCO was able to protect diabetic mice from atherosclerosis and renal disease.^{90,91} Various other in vivo studies supported the nephro- and retinoprotective effect of carnosine treatment in diabetic animals.⁹² However, in two of these studies, the protective effects of carnosine could not be explained by the anti-oxidative and anti-glycation characteristics of carnosine, but were exerted through other mechanisms.93,94

In clinical studies, carnosine derivative N-acetylcarnosine has been investigated as a treatment for (glycation-induced) cataract. Eyedrops with 1% N-acetylcarnosine (Can-C[™] Eye

Drops), which is more resistant to carnosinase than L-carnosine, improved vision in both subjects with cataract and without.⁹⁵ The effect of carnosine on the skin has been examined as well, since SAF increases during aging and correlates with AGE deposition in the skin.¹⁰ AGEs induce apoptosis of dermal fibroblasts and crosslinking of collagen, leading to stiffness of the tissue.⁹⁶ The subjects in this study were given supplements that combine L-carnosine with competitive carnosinase inhibitors to increase tissue L-carnosine levels without increasing its concentration in blood plasma (Can-C Plus, Innovative Vision Products, Inc., New Castle, DE, USA). Oral supplementation for three months had a positive influence on signs of skin aging, such as reduction of fine lines and improved skin appearance.⁹⁷

Polyphenols

Many nutraceuticals are rich in polyphenols that possess anti-glycation activity through various mechanisms, such as regulation of glucose metabolism, antioxidant effects and inhibition of the Aldose Reductase (AR) pathway.⁹⁸ AR is an enzyme in the polyol pathway and active/enhanced under hyperglycaemic conditions. The polyol pathway is an important source of diabetes-induced oxidative stress and the formation of reactive fructose and 3-DG that contribute to AGE accumulation.⁹⁹

Epigallocatechin 3-gallate

Epigallocatechin 3-gallate (EGCG) is the major polyphenol in tea that possesses anti-inflammatory and anti-cancer properties.¹⁰⁰ In a diabetic mouse model, induced by a high fat diet, EGCG proved to decrease AGE accumulation with 80% in kidney and 96% in heart. Furthermore blood glucose levels and weight gain induced by a high-fat diet were reduced and the formation of cataracts could be prevented or delayed.¹⁰¹ In another study mice were treated with low and high doses of EGCG three times a week. After seventeen weeks, mice receiving a high dose of EGCG showed a near reversal of weight gain, improved glucose control and inhibited AGE accumulation in plasma, liver, kidney and adipose tissue.¹⁰² In rats suffering from diabetic nephropathy, EGCG showed comparable results, ameliorating the decline of kidney function.¹⁰³

Additionally, green tea extract that is enriched with EGCG increased sRAGE levels in plasma of T2DM patients. sRAGE acts as a decoy for AGEs, preventing their interaction with RAGE.¹⁰⁴ Simultaneously, EGCG-rich green tea extract decreased RAGE ligand S100A12, which indicates EGCG is able to block RAGE-ligand signalling, and could possibly prevent the progression of inflammatory responses that lead to the complications in diabetes.¹⁰⁵

Although these results suggest the AGE inhibitory potential of the green tea derived EGCG, clinical studies are needed to show whether this could be a useful intervention for the treatment of diabetic complications. Examining the pharmacokinetics and safety issues is of great importance, since the dose used in in vivo studies far exceeds the amount of active substance in dietary supplements. Moreover, EGCG has been shown to have a low bioavailability, which led to the design of lipophilized derivatives that demonstrated slightly improved AGE-inhibitory activity in vitro.¹⁰⁶

Rutin

Rutin is a dietary flavonoid that is present in fruits, vegetables, tea and wine. Rutin is metabolized to a range of compounds such as quercetin, 3,4-DHT and 3,4-DHPAA. Rutin, along with its metabolites, can inhibit glucose autoxidation, the formation of AGEs and glycation of collagen.¹⁰⁷ Its anti-glycation capacity was again demonstrated on goat eye lens proteins, indicating rutins potential to scavenge free radicals and chelating metal ions. The inhibition of aldose reductase (AR) is another mechanism that explains the benefits of rutin.¹⁰⁸

The lowering effect of rutin on collagen fluorescence in diabetic rats was first described by Odetti et al.¹⁰⁹ Further in vivo studies demonstrated that G-rutin, a rutin glucose derivative, could reduce glycation of serum and kidney proteins and lipid peroxidation in diabetic rats.¹¹⁰ In addition, rutin had preventive effects on the development of diabetic nephropathy in rats. After 10 weeks not only the expression of AGEs and accumulation of collagen were significantly reduced, fasting glucose levels and oxidative stress were decreased as well. Furthermore, rutin treatment attenuated microalbuminuria and the thickness of the glomerular basement membrane.¹¹¹ Other studies examining the effect of rutin on rats with streptozotocin (STZ)-induced diabetes showed a protective function against kidney damage by positively regulating matrix remodelling¹¹² and improvement of hyperglycaemia and dyslipidemia, while liver and heart toxicity induced by diabetes were ameliorated.¹¹³ Hence, rutin supplementation might be a potential treatment for diabetic pathological conditions.

Resveratrol

Resveratrol has been described as a polyphenol that possesses anti-oxidant, anti-cancer, anti-inflammation and life extending effects.¹¹⁴ Besides these positive health effects, resveratrol showed the ability to inhibit AGE formation in vitro. Resveratrol acted as an inhibitor of α -amylase and α -glucosidase, which are enzymes that catalyse the degradation of carbohydrates.¹¹⁵ Inhibition of these enzymes modulates sugar release and postprandial hyperglycemia and could be used as a therapy to decrease the risk of diabetes complications.¹¹⁶

Two in vivo studies report that resveratrol treatment did not affect AGE levels in liver and kidney, which might be due to relative short exposure time. Nevertheless, resveratrol improved antioxidant status and decreased plasma glucose and RAGE expression in liver and kidney of diabetic rats.^{117,118} Moreover, resveratrol treatment reduces the NF-KB-RAGE signalling pathway, thereby ameliorating vasculopathy in diabetic rats.¹¹⁹ Furthermore, resveratrol can exert its beneficial effects on diabetic complications by inhibition of Aldose Reductase (AR), resulting in a decrease of AGE formation in the kidney and improvement of the glomerular filtration rate and renal function in diabetic rats. Since AR mediates the polyol levels that contribute to cataract formation, resveratrol is able to inhibit opacification of the lens.¹²⁰ Palsamy et al. support that resveratrol is able to normalize AR activity, and additionally other polyol pathway enzymes such as sorbitol dehydrogenase and glyoxalase-I (Glo-I), which limits AGE formation and glycative damage to the kidneys.¹²¹ In a clinical study the effect of resveratrol and hesperetin on vascular function was observed. These dietary bioactive compounds demonstrated to be strong inducers of glyoxalase-I, that is responsible for the detoxification of RCS compound MG. Co-therapy of resveratrol and hesperetin, instead of individual administration, was able to improve fasting plasma glucose, oral glucose insulin sensitivity and arterial and renal function in obese subjects.¹²²

Conclusions

AGEs build up in the body naturally during aging. Yet, excessive AGE accumulation can accelerate the aging process and induce tissue damage, which is most evident in tissues consisting of long-lived proteins such as skin, heart, muscles and joints. Furthermore, AGEs are key players in the development and progression of many chronic diseases such as diabetes mellitus, cardiovascular disease and renal disease, and their complications. Since AGE levels could represent biological age and have shown to be a good predictor of cardiovascular and diabetes complications, AGE measurements can provide new information about a subject's health or to the current prognosis of diabetic patients. Consequently, the investigation of anti-AGE interventions has been of major interest the latest years. The complexity of AGE formation and interactions allow for interventions at different levels. The strategies that prevent AGE formation depend on different mechanisms such as antioxidant ability, scavenging of reactive carbonyl species and inhibition of aldose reductase. Pharmaceutical options show promising results, yet their clinical relevance is doubtful so far due to safety concerns. For individuals with high AGE levels but no clinical symptoms other interventions might be more effective. Lifestyle interventions such as a low AGE diet and physical exercise are easily to implement and show beneficial effects on plasma AGEs and reduce inflammation and oxidative stress. Nutraceuticals, derived from food sources and available

as dietary supplements, have mostly been investigated in preclinical studies. Many studies evaluated these compounds as potential anti-AGE therapeutics and showed positive effects on diabetic complications such as nephro- and retinopathy. Until now, clinical evidence is mostly lacking and should be evaluated in the future. The duration of the interventions that is required to show a beneficial effect on AGEs is uncertain and might depend on the part of the AGE formation pathway the intervention is aimed at. Due to the fact that AGE accumulation represents glycaemic memory, interventions against AGEs could have prolonged effects on health and prevention of diabetic complications.

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