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Advanced glycation end-products (AGEs) and glycometabolic and oxidative status in overweight subjects: an application of skin autofluorescence

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ABSTRACT

Introduction: Overweight and obesity increase the risk of mortality following the onset of several diseases generally characterized by oxidative stress. The levels of advanced glycation end-products (AGEs), a consequence of metabolic disorder and oxidative stress, play an important role in the process, and their quantification, based on skin autofluorescence (skin AF), could be used for non-invasive assessment of AGEs.

Aim: To evaluate in overweight subjects the diagnostic use of AGE determination (skin AF detected by an AGE Reader) for assessing possible correlation between AGEs and some anthropometric/oxidative indices.

Patients and methods: 51 consecutive overweight participants in a nutritional education programme were enrolled in this observational cross-sectional study: 39 women (aged 49.69±13.71; BMI 33.12±5.44 kg/m²) and 12 men (aged 56.84±17.84; BMI 33.12±3.11 kg/m²). Glycometabolic and oxidative parameters were measured using routine laboratory analyzers. Pearson's correlation coefficient was used for statistical analysis.

Results: Significant correlations were found between the Cardiovascular Risk Index and age (r=0.65; $p<0.0001$), AGEs (r=0.41; $p<0.0001$) and glycosylated haemoglobin (r=0.38; $p<0.05$); ageing and AGEs (r=0.50; $p<0.0001$) and glycosylated haemoglobin (r=0.40; $p<0.0001$); and C-reactive protein and fibrinogen (r=0.52; $p<0.0001$), homocysteine and fasting glucose (r=0.47; $p<0.0001$).

Discussion: Oxidative stress can be assessed by AGE determination. Our findings in overweight subjects highlight interesting correlations between metabolic-oxidative parameters. Age emerged as the most important indicator of cardiovascular risk and AGE formation. Notably, skin AF, detected by the AGE Reader (a simple non-invasive clinical tool), can be a useful marker for rapid assessment of dysmetabolic-oxidative risk in overweight subjects.

Keywords

Skin auto-fluorescence (SAF)

AGEs

Advanced glycation endproducts

Overweight

Oxidative status

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Introduction

Obesity, one of the greatest public health challenges of the 21st century, has been defined by the World Health Organization (WHO) as a condition characterized by a body mass index (BMI) above 30 kg/m^2 and excessive fat accumulation affecting health [1]. Overweight and obesity increase the risk of death following the onset of other diseases such as type 2 diabetes, hypertension, heart disease, glucose intolerance, insulin resistance and hyperlipidaemia characterized by high levels of cholesterol and/or triglycerides and/or low HDL levels [2].

Many pathological conditions associated with obesity are characterized by oxidative stress caused by the oxidation of lipids, proteins and DNA by reactive oxygen species such as superoxide anions, hydroxyl radicals and hydrogen peroxide [3–6].

Moreover, increasing evidence has revealed the important role of advanced glycation end-products (AGEs), caused by metabolic disorders and oxidative stress, in atherosclerosis and diabetes [7]. From a chemical point of view, increased blood glucose, caused by insulin deficiency and/or insulin resistance, reacts via its carbonyl group with the amino group in proteins to form glycosylated proteins which are also related to normal ageing. Glucose autoxidation by reactive oxygen species is another pathway leading to AGE formation [8].

AGEs play an important role in the development of chronic age-related diseases (i.e., diabetes and cardiovascular diseases) and can offer prognostic information: their levels in tissues are considered to provide a 'human metabolic memory' closely related to inflammatory markers and oxidative stress. They can be non-invasively assessed by skin autofluorescence (AF) assessment and are useful for possible associations with other indices [7–10].

AGEs are the result of, and therefore provide information on, glycometabolic and oxidative stress leading to the development of vascular complications through two pathways: binding to AGE receptors and cross-linking to proteins. Therefore, AGEs can be used as a marker of cardiovascular disease, diabetic complications and neurodegenerative disorders. Moreover, AGEs accumulate throughout the entire body including the skin where they cross-link to collagen and elastin leading to the development of wrinkles, lack of elasticity and older-looking skin. Other AGE sources are heating of food and smoking [11]. The AGE Reader can easily measure AGEs in the skin by evaluating AGE autofluorescence [11], but it is challenging to determine to what extent inflammation and oxidative markers are influenced by food components or nutrients [12].

Over the past two decades, an increasing number of studies have focused on glucose and oxidative status involved in a wide range of chronic diseases, inflammatory/immune disorders, nutritional status, the influence of nutrients on body function and well-being [3, 13, 14].

The present study was aimed at evaluating the diagnostic use of AGE determination to assess possible correlations between AGEs and some anthropometric/biochemical indices in overweight subjects.

Subjects and methods

This observational cross-sectional study enrolled 51 consecutive participants in a nutritional education programme organized by the Occupational Medicine Department, Fondazione IRCCS Ca' Granda, Ospedale Maggiore, Italy. The study subjects included 39 women (aged 49.69±13.71; BMI 33.12±5.44 kg/m^2) and 12 men (aged 56.84±17.84; BMI 33.12±3.11 kg/m^2).

At the beginning of the study, each subject attended a clinical consultation during which routine measurements, a medical history and nutritional information were collected. All participants gave informed consent. Exclusion criteria included pregnancy or lactation, chronic skin disease (i.e., lupus), skin phototype 3 or 4, chronic medication, oral contraceptives, gastrointestinal disorders, unstable psychiatric state, alcohol abuse, or use of dietary supplements.

Anthropometric measurements and impedance were recorded using a body composition analyzer (InBody2000; InBody, Seoul, South Korea), and body mass index (BMI) and the percentage of fat mass were determined. BMI was calculated as the ratio between weight (kg) and height (m^2) and categorized, according to the standard classification of the WHO, into overweight (BMI 25.0–29.9 kg/m^2) and obesity (BMI >30.0 kg/m^2). Obesity was further classified into three groups according to its severity (class I: BMI 30.0–34.9 kg/m^2; class II: BMI 35.0–39.9 kg/m^2; and class III: BMI >40.0 kg/m^2) [1].

Systolic and diastolic blood pressure was also recorded.

Routine biochemical parameters (glucose, insulin, creatinine, triglycerides, cholesterol, HDL, LDL, AST, ALT, GGT, homocysteine, C-reactive protein and uric acid) were measured on fasting serum samples using a Modular analyzer (Roche, Basel, Switzerland). Plasma glycosylated haemoglobin levels were assessed by HPLC. Complete blood count and fibrinogen were determined by routine laboratory analyzers.

The Homeostasis Model Assessment of Insulin Resistance index (HOMA-IR) was calculated as previously described by Matthews *et al* [15] using the formula: [insulin (μU/ml)×glucose (mmol/l)]/22.5.

The Cardiovascular Risk Index for subjects aged 35–69 was assessed according to sex, age, smoking habit, systolic blood pressure, total cholesterol, HDL cholesterol, and hypertension and anti-hypertensive medication by means of software used by 'The Heart Project' of the Superior Institute of Health ('Il Progetto Cuore' of the Istituto Superiore di Sanità), Rome, Italy [16].

The study was conducted in accordance with the principles stated in the Declaration of Helsinki for Research on Human Subjects and was approved by the local ethics committees (study registration number: 1370).

Figure 1 - AGE Reader (Diagnoptics Technologies, Groningen, The Netherlands)

Measurement of AGEs

The autofluorescence of AGEs in the skin was measured three times with an AGE Reader (Diagnoptics Technologies, Groningen, The Netherlands) connected to a spectrometer with a fluorescence detector (Fig. 1) and to a computer. This instrument can measure fluorescence from AGEs in skin collagen rapidly, simply and non-invasively [11]. The autofluorescence measurements are calculated in arbitrary units (AU) and obtained by dividing light intensity in the 420–600 nm range by light intensity in the 300–420 nm range [17].

Statistics

The relationships between anthropometric indices and quantitative variables were analysed using Pearson's correlation coefficient and GraphPad Prism 5.00.288 software. All data were expressed as the mean±standard deviation (SD) and range (minimum–maximum). The intra-assay and inter-assay variation coefficients were 10% and 15%, respectively. Correlation graphs were drawn using SAS 9.3 software.

Results

Table 1 shows the anthropometric and biochemical parameters of the 51 overweight subjects.

Table 2 shows Pearson's correlation coefficient between the variables. The Cardiovascular Risk Index showed significant positive correlations, which are stronger with age (r=0.65; $p<0.0001$) than with glycosylated haemoglobin (r=0.38; $p<0.05$; Fig. 2) or AGEs (r=0.41; $p<0.0001$; Fig. 3).

Glycosylated haemoglobin showed significant positive correlations which were weak with mean AGEs (r=0.35; $p<0.0001$; Fig. 4) but strong with fasting glucose (r=0.74; $p<0.0001$; Fig. 5); moreover, mean AGEs correlated with fasting glucose (r=0.47; $p<0.0001$; Fig. 6).

Parameters	Mean±SD	Range
Age (years)	51.37±14.90	17.93–75.13
BMI (kg/m²)	33.24±4.97	25.57–45.62
AGEs	2.16±0.55	1.27–4.23
Norm AGEs to age	4.47±1.46	2.25–8.35
Systolic blood pressure (115–120 mmHg)	121.37±14.60	95–155
Diastolic blood pressure (75–80 mmHg)	76.76±6.84	60–90
Fibrinogen (165–350 mg/dl)	286.16±62.22	172–464
C-reactive protein (<0.5 mg/dl)	0.38±0.44	0.04–2.55
Uric acid (2.4–7.0 mg/dl)	5.14±1.30	3.10–9.70
Creatinine (0.50–1.20 mg/dl)	0.73±0.13	0.49–1.00
Triglycerides (<170 mg/dl)	107.41±41.69	48–208
Total cholesterol (<200 mg/dl)	204.04±48.19	112–357
AST (5–38 U/l)	20.18±5.28	12–37
ALT (5–41 U/l)	22.51±10.42	6–48
Gamma-GT (5–61 U/l)	24.25±28.85	7–203
Homocysteine (<10.5 μmol/l)	11.52±4.01	6.10–30.50
Fasting glucose (65–100 mg/dl)	96.84±12.37	69–133
Fasting insulin (2.6–25 μIU/ml)	14.72±8.45	4.26–40.87
Glycosylated Hb (20.0–42.0 mmol/mol)	35.80±4.53	27–45
HOMA-IR	3.61±2.20	0.86–9.79
Cardiovascular Risk Index (<3%)	2.63±2.77	0.20–11.50

Values in brackets are reference intervals or cut-off values
Norm AGEs, age-normalized advance glycation end-products

Table 1 - Anthropometric and biochemical parameters of study subjects

Variable 1	Variable 2	r (Pearson)	*p* Value
Cardiovascular Risk Index	Age (years)	0.65	<0.0001
	Glycosylated haemoglobin	0.38	<0.05
	Mean AGEs	0.41	<0.0001
C-reactive protein	Fibrinogen	0.52	<0.0001
HOMA-IR	Glycosylated haemoglobin	0.41	<0.0001
	Fasting insulin	0.93	<0.0001
	Fasting glucose	0.58	<0.0001
	Mean AGEs	0.35	<0.0001
Glycosylated haemoglobin	Fasting glucose	0.58	<0.0001
	Mean AGEs	0.50	<0.0001
Homocysteine	Fasting glucose	0.47	<0.0001
Mean AGEs	Fasting glucose	0.47	<0.0001
Age	Mean AGEs	0.50	<0.0001
	Glycosylated haemoglobin	0.40	<0.0001

Table 2 - Pearson's correlation coefficient between the variables considered

Other significant positive correlations were found between age and glycosylated haemoglobin (r=0.40; $p<0.0001$; Fig. 7) and between age and AGEs (r=0.50; $p<0.0001$; Fig. 8). Ageing was possibly more correlated with AGEs than with glycosylated haemoglobin. Significant positive correlations were also observed between C-reactive protein and fibrinogen (r=0.52; $p<0.0001$) and between homocysteine and fasting glucose (r=0.47; $p<0.0001$).

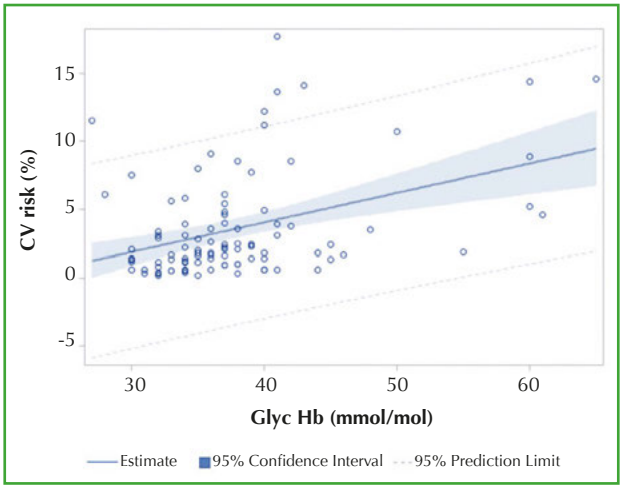

Figure 2 - Correlation between glycosylated haemoglobin (abscissa) and cardiovascular (CV) risk (ordinate)

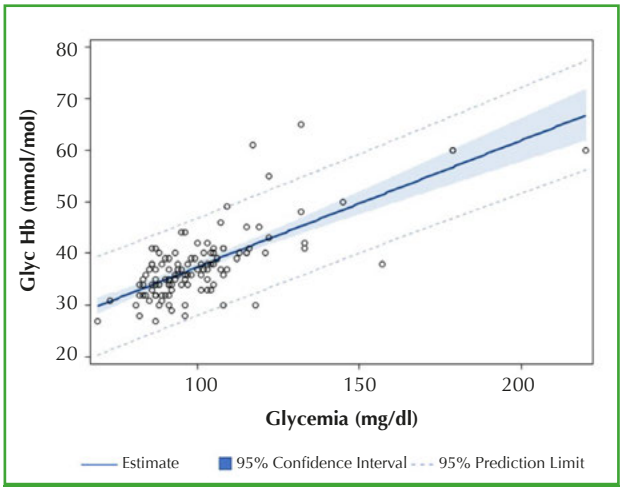

Figure 5 - Correlation between glycaemia (abscissa) and glycosylated haemoglobin (ordinate)

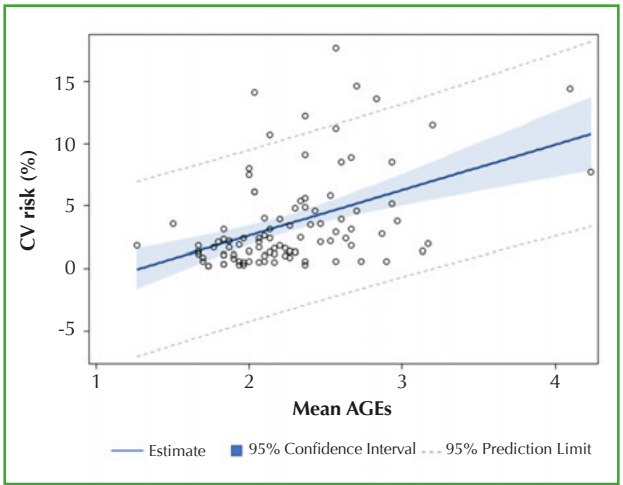

Figure 3 - Correlation between mean advanced glycation end-products (AGEs) (abscissa) and cardiovascular (CV) risk (ordinate)

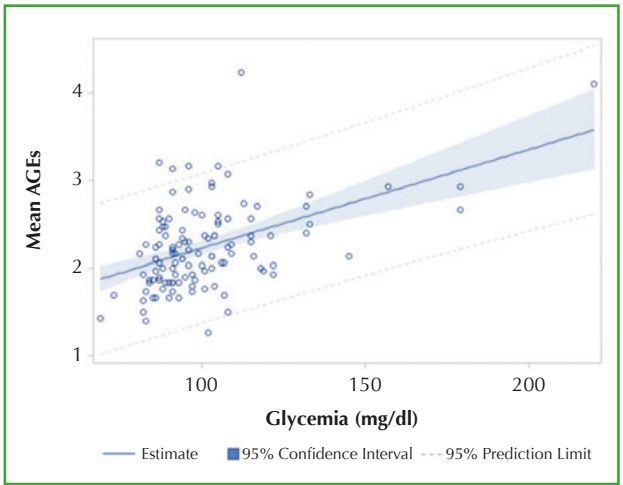

Figure 6 - Correlation between glycaemia (abscissa) and mean advanced glycation end-products (AGEs) (ordinate)

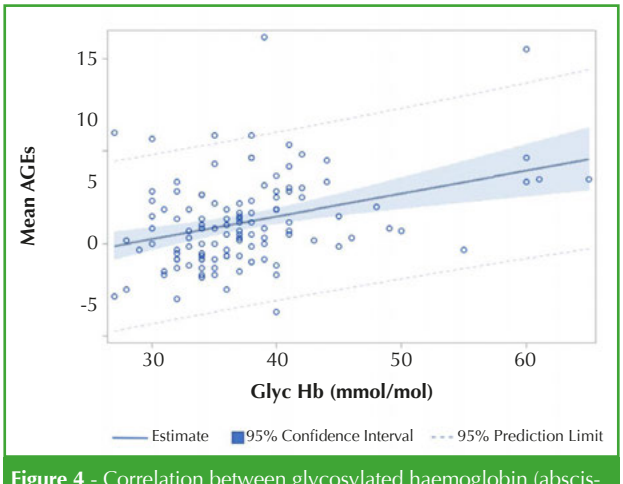

Figure 4 - Correlation between glycosylated haemoglobin (abscissa) and mean advanced glycation end-products (AGEs) (ordinate)

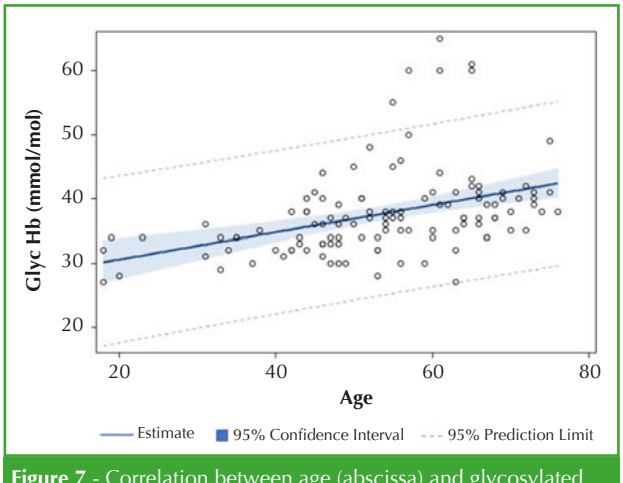

Figure 7 - Correlation between age (abscissa) and glycosylated haemoglobin (ordinate)

As expected, significant correlation was found between HOMA-IR (for quantifying insulin resistance) and fasting insulin (r=0.93; $p<0.0001$), which was higher than those between HOMA-IR and the other indices (i.e., glycosylated haemoglobin, fasting glucose, mean AGEs).

Discussion

Based on these preliminary findings, the present study highlights interesting correlations between metabolic-oxidative indices, ageing/cardiovascular risk and AGEs in overweight

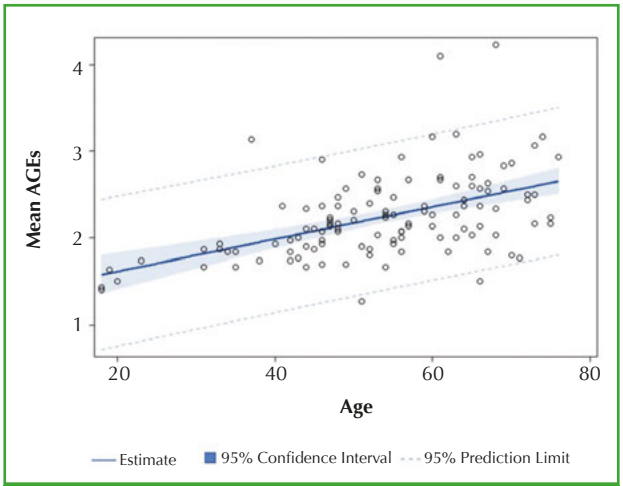

Figure 8 - Correlation between age (abscissa) and mean advanced glycation end-products (AGEs) (ordinate)

subjects. AGE accumulation in tissue, by promoting self-, glucose and/or lipid oxidation, reflects changes in oxidative state causing several AGE-related chronic diseases [7, 8]. We describe the use of a new biological non-invasive marker (skin AF detected by an AGE Reader [17]), and the diagnostic use of the AGE determination and correlations between AGEs and some anthropometric/biochemical indices [9–11, 18].

Obesity is a chronic degenerative disease characterized by a change in body composition caused by fatty mass due to excessive calorie consumption compared to energy expenditure. Our findings in overweight subjects highlight significant positive correlations between the indices evaluated, among which age seems to be the most important non-modifiable factor increasing both cardiovascular risk and AGE accumulation.

Other researchers have found significant high correlation between the Cardiovascular Risk Index and age, an unmodifiable risk factor [19], as arteries stiffen with age and, consequently, blood pressure rises. Other significant, although slightly weaker correlations, were also observed between two indices of inflammation and possible cardiovascular risk (C-reactive protein and fibrinogen) [20], and between homocysteine and fasting glucose [21], both indices correlated with cardiovascular risk. Homocysteine is a proven independent cardiovascular risk factor for cardio- and cerebrovascular disease [21, 22], while glucose change preceding diabetes is connected with both micro- and macro-vascular complications [23]. Another significantly positive correlation, although again slightly weaker, was observed between glycosylated haemoglobin and HOMA-IR [24], which were both positively correlated with glucose and insulin. HOMA-IR showed a more positive correlation with fasting insulin (r=0.93; $p<0.0001$) than with fasting glucose (r=0.58; $p<0.0001$), while glycosylated haemoglobin was more positively correlated with glucose [24] than with HOMA-IR. The HOMA model was used to determine insulin sensitivity and β-cell function from fasting plasma insulin and glucose concentrations. The relationship between glucose and insulin in the basal state reflects the balance between hepatic glucose output and insulin secretion, which is maintained by a feedback loop between the liver and β-cells [25].

HOMA-IR reflects the lack of insulin receptors and therefore insulin resistance, whereas glycosylated haemoglobin reflects changes in glucose in the preceding 3 months.

Recently, the important role of AGEs, which develop as a consequence of metabolic disorders and oxidative stress, in atherosclerosis and diabetes has been reported [7]. AGEs can provide prognostic information concerning the development of chronic age-related disease, such as diabetes and cardiovascular disease, and their tissue levels are considered to provide a 'human metabolic memory' closely related to markers of inflammation and oxidative stress.

A significantly positive correlation, although slightly weaker, has been observed between glycosylated haemoglobin and AGEs, with both oxidative stress indices representing a metabolic memory and both being positively correlated with fasting glucose. AGEs and glycosylated haemoglobin are influenced by glycometabolic status, with glycosylated haemoglobin being the main index of blood glucose changes. Glycosylated haemoglobin and AGEs are in turn correlated with ageing and the Cardiovascular Risk Index [26]. Ageing is more strongly correlated with AGEs [27] than with glycosylated haemoglobin [28, 29]. Both these parameters are indicative of metabolic and oxidative stress, which increases with age [29]. Analogously, the Cardiovascular Risk Index showed higher correlation with AGEs [30] than glycosylated haemoglobin. AGEs are considered a new biological marker of oxidative stress which increases in chronic disease (i.e., diabetes) as a result of glucose auto-oxidation and lipo-oxidation [30] pathways whereby AGEs are formed through the action of reactive oxygen species [8].

Overweight and obesity, chronic degenerative diseases characterized by a change in body composition, increase the risk of death following the onset of several other diseases. The findings of our study in overweight subjects have highlighted positive correlations between the indices evaluated. Notably, age emerged as the most important non-modifiable risk factor for cardiovascular disease and AGEs. AGEs, the 'body's metabolic memory' [7], are considered a measure of metabolic and oxidative stress. These glycation products ac-

cumulate normally during old age but more rapidly in some conditions (i.e., tissue with micro- and macro-vascular damage, obesity, diabetes, atherosclerosis, Alzheimer's disease, vascular dementia, kidney disease, rheumatoid arthritis, sarcopenia, osteopenia, degenerative eye diseases, Parkinson's and other chronic diseases) [31].

The current study shows that the AGE Reader (a non-invasive clinical tool) can be used to determine skin AF which can be a useful marker of tissue AGE accumulation and, consequently, AGE-related diseases.

AGEs can be ingested with food, in which case they are called dietary advanced glycation end-products (dAGEs) [31]. Dry heating of some types of cooked food, particularly lean red meat and poultry, causes dAGEs to form. Food rich in carbohydrates (i.e., vegetables, fruit, cereals, legumes) also contain small quantities of dAGEs once cooked [32]. Some studies indicate that the simultaneous presence of different factors such as the AGE inhibitory compound aminoguanidine, cooking at a lower temperature and shorter cooking times together with exposure to acid solutions (marinades) prevents the formation of dAGEs in cooked meat [32]. An experiment was conducted on some samples of lean meat marinated in an acidic solution of either vinegar or lemon juice for 1 hour before cooking: the ascorbic acid in the lemon juice helped to reduce the glycation process [32].

As oxidation steps are crucially involved in the formation of many AGEs, substances with antioxidative or metal chelating properties have antiglycating activity [33, 34]. Consequently, increasing interest has been directed to nutrients and vitamins, so-called 'nutraceuticals', and other dietary supplements for use as natural tools against AGEs [33, 35, 36]. The use of active ingredients of vegetable or animal origin has been proven to have beneficial health effects and to be a possible alternative method for preventing many pathological conditions [37, 38] as shown by the two following studies.

Long-term cigarette smoking has negative effects on oxidative status as it promotes the oxidation of lipids, proteins and DNA and the formation of AGEs. The effect of nutraceutical supplementation, consisting primarily of mixed juice powder concentrates, on the oxidative status of healthy heavy smokers was evaluated. The 3-month intervention with a nutraceutical formulation reduced some oxidative alterations attributed to long-term cigarette smoking [39].

Recently, a nutraceutical formulation containing polyphenolic extract of the Annurca apple has been tested in a clinical trial on healthy subjects affected by mild hypercholesterolaemia; administration of capsules for 12 weeks showed a good hypocholesterolaemic effect [40].

Alpha-lipoic acid, one of the best studied nutraceuticals, is able to reverse tail tendon collagen glycation in fructose-fed rats, an effect that is attributed to its endogenous antioxidant action and to its ability to recycle ascorbic acid, alpha-tocopherol and GSH [33]. An interesting study evaluated the effects of an oral liquid formulation of the natural and more active alpha-lipoic acid enantiomer on the oxidative status of 20 subjects. After 1-month treatment, the formulation seemed to strengthen the endogenous antioxidant barrier and, interestingly, helped mitigate the pain typical of oxidative-dependent disease [41].

Finally, AGE accumulation and AGE-related consequences are important targets for the skincare industry since AGEs also cause wrinkling of the skin: most large international cosmetic companies have already developed skin creams and capsules targeting AGEs to reduce skin wrinkles.

Conclusions

This study highlights the usefulness of a new biological non-invasive marker (skin AF) and the interesting correlations between metabolic-oxidative indices, ageing/cardiovascular risk and AGEs. Tissue AGE accumulation, by promoting self-, glucose and/or lipid oxidation, reflects changes in oxidative state, which can cause several AGE-related chronic diseases. Notably, skin AF, detected by the AGE Reader, is possibly a very useful marker for rapid assessment of dysmetabolic-oxidative risk in overweight subjects. Lifestyle (dietary program, nutraceutical food supplements and increase in physical exercise) changes help improve glucose control, encourage weight loss and promote well-being, all of which are associated with lower levels of tissue AGE accumulation.

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