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Advanced glycation end products as biomarkers in systemic diseases: Premises and perspectives of salivary advanced glycation end products

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Title Page Template

- **Article title: Advanced Glycation End Products (AGEs) as biomarkers in systemic diseases**
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Advanced Glycation End Products (AGEs) as biomarkers in systemic diseases

Abstract: Advanced glycation end products (AGEs) are glycated proteins associated with high dry temperature food processing, coloring and flavor modification of food products. Previous studies on diet-related disease support the role of the glycation products as biomarkers in local and general pro-inflammatory response. Exogenous and endogenous AGEs are involved in chronic low-level inflammation, which underlies the onset of metabolic syndrome influenced by food intake, there by demonstrating their implication in diet-related pathologies. Although studies have revealed a strong association between the accumulation of AGEs and the occurrence/worsening of metabolic diseases, their routine use for the diagnosis or monitoring of local and general disease has not yet been reported.

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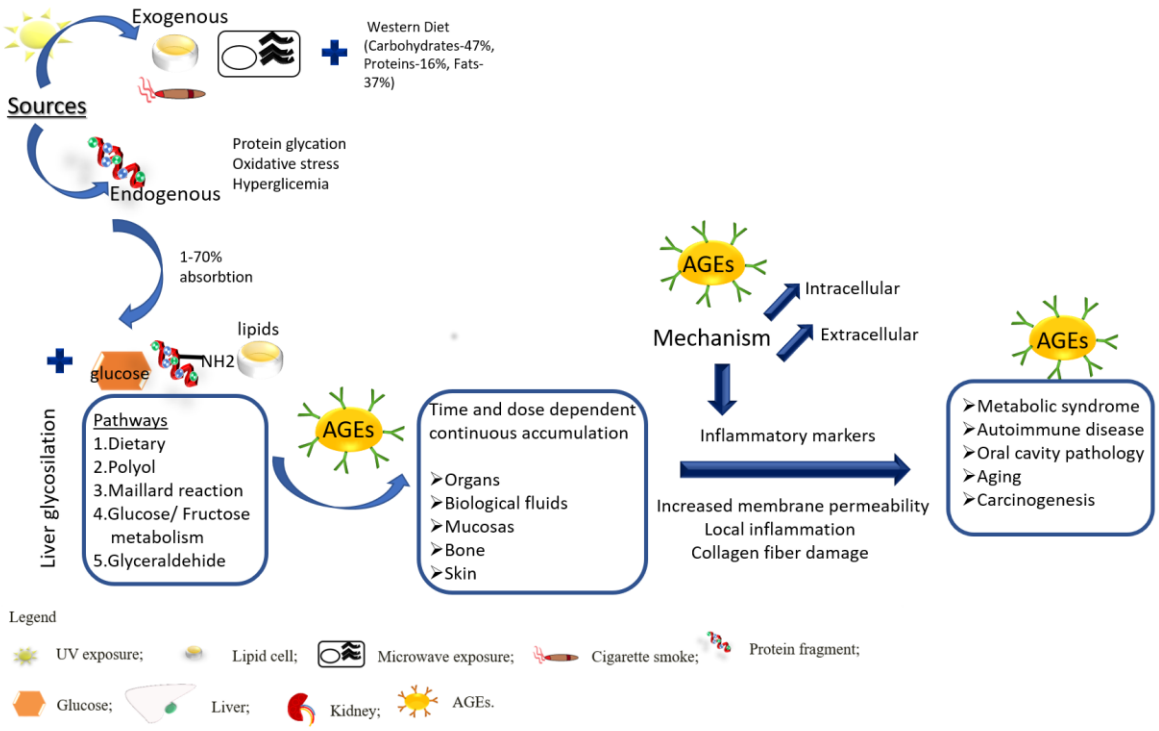

Keywords: Advanced glycation end products – AGEs; chronic low level-inflammation; meta-inflammation, metabolic syndrome; diet-related disease; AGEs diagnosis and downregulation; biomarkers

Article Body Template

- **Main body of text:**
- **Introduction**

AGE production sources, chemistry, and metabolism

Advanced glycation end products (AGEs) were synthesized for the first time *in vitro* in 1912, by French Louis-Camille Maillard; later, in 1953, John E. Hodge divided the reaction, and in 1986, the formation of reducing sugar-derived carbonyl products was added to the Maillard chain reaction [1, 2]. AGEs are represented by structures formed via glycation- a non-enzymatic reaction (Van Nguyen, 2006) between sugars with a free amino or ketone group and proteins or lipids. This complex and difficult to control reaction is involved in food taste and color modification, especially during processes such as baking, toasting, and almost all animal protein cooking processes. In medical research, AGEs have been associated with increased production of ROS (reactive oxygen species), which consequently generate oxidative or carbonyl stress, with negative effects in inflammatory, autoimmune, and diet-related diseases.

In the present narrative paper, aspects concerning AGEs sources, mechanism of action, the implication in systemic and diet-related disease, and the aging process as a subsection will be discussed. A current approach based on salivary AGEs as an inflammatory process biomarker will be presented, as well as anti-AGEs downregulation strategies, tested *in vitro* either *in vivo*.

AGEs sources

AGEs can be classified according to their provenience, fluorescence and crosslinking ability. One of the classification [3] include fluorescent AGEs such as GOLD, glyoxal-lysine dimmer, GOLDIC, (2-ammonio-6-([2-[(4-ammonio-5-oxido-5-oxopentyl)amino]-4,5-dihydro1H-imidazol-5-ylidene]amino)hexanoate), MOLD, methyglyoxal-lysine dimmer; MODIC, (2ammonio-6-([2-[(4-ammonio-5-oxido-5-oxopentyl)amino]-4-methyl-4,5-dihydro-1Himidazol-5ylidene]amino)hexanoate); MRX, 8-hydroxy-5-methyldihydrothiazolo(3,2-alpha)pyridinium-3carboxylate, pentosidine, crossline and non-fluorescent non-crosslinked AGEs such as carboxymethyllysine- CML, carboxyethyllysine, pyrraline, imidazole. AGEs sources can be endogenous and exogenous.

Endogenous AGEs are generated by protein glycation due to oxidative stress and hyperglycemia, which are commonly associated with diabetes mellitus (HbA1c- glycosylated hemoglobin). In hyperglycemia, glucose converts to fructose through an enzymatic process. Under aldosereductase and sorbitol dehydrogenase reaction, fructose accumulates in tissues and decreases glyceraldehyde-3-phosphate (GAP) activity, thus enhancing augmentation of glyoxal (GO) and methylglyoxal (MGO) AGEs precursors GAP and Dihydroxyacetone phosphate (DHAP) [4]. It was observed (Rahbar, Blumenfeld and Ranney, 1969) that protein glycation takes place in the human body similarly with processed food, leading to glycated hemoglobin.

Carbonyls and dicarbonyls groups from thermally prepared food are found in a high percentage in human tissues [5].

The exogenous sources include nutrients- dietary AGEs (dAGEs), UV (ultraviolet) exposure, cigarette smoke compounds, microwaves and ultrasounds. DAGEs are easily obtained by ingesting nutrients that are part of the Western diet (carbohydrates - 47%, lipids - 37%, protein compounds - 16%). Nutrients rich in lipids and proteins have higher AGEs concentration compared to carbohydrates [4]. Regarding the temperature and cooking method on dAGEs, dietitians [6] evaluated CML as an AGEs biomarker in 250 sorts of nutrients, using the next preparation methods: boiling (100°C), frying (180°C), broiling (225°C), roasting (177°C) and oven frying (230°C). In the evaluated food, CML levels were highest in oven frying, followed by deep frying, roasting and water boiling. Besides the cooking conditions, time is also an important factor in the amount of resulting glycation products. Helou et al. [7] showed that bread baked at 250°C for 9 minutes led to 19,6% more CML compared with 200^0 C for 12 minutes. Microwave heating significantly increased CML production in saccharide-lysine model systems compared with boiling heating [8]. It was estimated that in European patients, one day melanoidins products intake variates from 10 to 12 grams per day from all sources, where coffee and bread are considered to represent half or more [9,10]. Compared to other sources, the exogenous glycation compounds provided by nicotine / nornicotine- smoking AGEs are formed in a couple of hours [11]. Subsequently, they bind to connective tissue collagen fibers and low-density lipoproteins (LDL) [12].

The glycation products can have a low molecular weight (LMW) or high molecular weight (HMW), formed in the advanced stages of the Maillard reaction [13]. A study conducted by Malene W. Poulsen et al. on a group of 36 rats treated with HMW and LMW dAGEs showed that LMW dAGEs were found in a higher concentration in biological fluids and organs [14]. Because protein-bounded dAGEs are evidenced after digestion, HMW and LMW compounds can be evaluated as precursors of melanoidins [15].

- **DAGEs metabolism**

Scheijen et al., in their study, developed a dAGEs database by identification of CML, Nepsilon-(carboxyethyl)lysine (CEL) and 5-(5-Hydro-5-methyl-4-imidazolon-2-yl) L-Ornithine d6 (MG-H1) in 190 different nutrients, and then correlated dAGEs with plasma and urinary levels [16]. They found a weak connection between dAGEs and protein bound plasma AGEs. Birlouez-Aragon's research suggested that when metabolized, dAGEs are divided to non-glycated proteins and free-glycated proteins, from which dAGEs are found circulating in their free form and thus, eliminated through urine [17]. This study is in assent with Mei Li's research [18], who studied the bioavailability of oral gavage pure CML on rat model. The authors found that dietary CML, after entering into circulation, form CML bound-proteins, which accumulate in organ tissue. Metabolic studies [19,20] showed that plasma AGEs in diabetes and plasma CML levels in renal failure decreased by 30%-40% when subjects were on a low glycotoxin diet.

The Dresden Institute for Food Chemistry [21] conducted an experiment in a group of 18 healthy subjects to study the bioavailability and elimination of fructoselysine, pyrroline, and pentosidine AGEs. The research team found that free urinary pyrroline and Amadori products had a dietary origin, while free pentosidine, endogenous *in vivo* formation should be taken into consideration. Alamir and coworkers [22] analysed on a rat model the dietary CML (derived from extruded / non-extruded casein) metabolism. The results showed no differences in plasma CML depending on CML origin; in urine and feces, extruded proteins levels were lower (38-48%) compared with non-extruded proteins (23-37%). A study that measured furosine accumulation in skin collagen showed that a restrictive diet could lead to limitation of early glycation Amadori products, depending on glycemia [23]. Another study that analyzed age related skin AGEs for 25 months [24], showed a reduction from 18% to 33% in diet-related early glycation AGEs. The accumulation of advanced CML and pentosidine decreased after a few months from the beginning of trial. These trials showed that long term AGEs depositing depended not only on the circulating sugars, but also on ROS and lipid oxidation. Although glycation compounds as melanoidins or others rest in the lower gastrointestinal tract for several hours [25], the low bioavailability of dAGEs is explained by formation of aggregates, cross-link reactions and the lack of specific transport systems [26].

Regardless of the source of glycation, the hepatic formation of AGEs occurs in several steps (Figure 3). From the dAGEs ingested, approximately 10% are intravasculary absorbed, from which 30% are urinary excreted. Koschinsky et al. performed a 3-day trial to analyze bioavailability of dAGEs in diabetic and healthy subjects, with or without renal dysfunction. Their results showed that approximately 10% of the ingested AGEs were found intravascular [27]. Urribari et al. performed a 3-day dietary restriction on dAGEs intake in a sub-group of 5 healthy subjects to analyze dAGEs metabolism. The results showed a 30-40% decrease in AGEs serum levels [4]. Other studies [26, 28] demonstrated that AGEs bypasses absorption due the resistance to hydrolysis of the crosslink bindings in the gastrointestinal tract.

- **DAGEs formation**

As mentioned above, Maillard reaction occurs in different cooking methods that are accountable of nutrients color, flavor and attractive appearance. The chemical reaction is between the carbonyl group of a reducing sugar and the amino group of the amino acid. At high temperatures, Amadori compounds are formed, firstly as stable Maillard reaction products. Of these, furosine is used to measure early glycation, formed after acid hydrolysis of the Amadori products fructosyl-lysine, tagatosyl-lysine and lactulosyl-lysine produced by the reaction of lysine with lactose, glucose, and galactose [29,30]. The final reactions are represented by condensation of sugar and proteins to polymeric melanoidinic compounds, from which CML is the most representative and a stable product, while GO and MGO present reactive activity [31].

Uribarri and his coworkers mentioned that exposure of uncooked food to barbecuing, grilling, roasting, baking, frying, sautéing, broiling, searing and toasting leads to acceleration of dAGEs formation [32]. Moreover,

dry heat cooking could cause formation of 10% to 100% new dAGEs. AGEs content (CML exactly) as kilounits/100 grams was analyzed in 549 foods. The study showed that high-heat treated meat contained the highest AGEs levels, followed by high-fat and conserved cheese [6]. Animal derived foods also have high AGEs levels, due to the continuous exposure to pasteurization or depositing at room temperature. Carbohydrates, which are rich in water and non-reducing sugars, have a lower expression of AGEs. These statements show that glycation occurs in a high amount in food components. Thoroughly research brought LC-MS (liquid chromatography–mass spectrometry) as evaluation method for the quantification of dAGEs. There have been validated several databases containing dAGEs ratio (nanogram per gram) [33,34].

Figure 2. AGE sources, metabolic pathways and effects on storage organs

- **AGEs pathogenic mechanisms**

AGEs can hurt tissue by two different pathogenic pathways: receptor-mediated and receptor-independent manner. One receptor for AGEs, known as RAGE, is a multi-ligand immunoglobulin family cell surface molecule, which possesses a 35-kDa polypeptide with a unique sequence at the NH_2-end and HMGB1 (high mobility group box 1)- a chromatin protein, and is the best characterized AGE receptor [35]. The receptor mechanism consists of binding to specific RAGE or immune Toll-like receptors (TLRs), expressed in macrophages, fibroblasts, epithelial and endothelial cells [36]. Other AGE receptors such as AGE-R1, AGE-R2, AGE-R3 and scavenger receptors (which facilitate protein absorption and degradation) [37] may have a role in extracellular AGE clearance by endocytosis and degradation. The non-receptor mechanism includes cross-linking of protein strands, forming strong covalent bonds, which reduce the elasticity and thus, the functions of proteins [38]. Once formed, they attract and activate inflammatory cells and enzymes; they mediate ROS generation. Increased membrane permeability and chronic inflammation confirm the key role of AGEs as common features linking metabolic abnormalities, inflammatory signaling and cardiovascular dysfunction in cardio-metabolic disorders. DAGEs increase tissue hardness and alter serum LDL levels. Their high serum ratio activates cytokines and ROS production, resulting in a strong association with the risk of aggravating existing diseases, and promotes the development of new pathologies (Figure 3).

Figure 3. The AGEs' negative effects on the human body

- **AGEs and the aging process**

AGEs are considered to be a biomarker for aging, due to the continuous accumulation in body fluids and tissues. AGEs are thought to have a role in aging because they accumulate in collagen and elastin fibers in the extracellular matrix, affecting its elasticity. AGEs alter the intracellular function by protein glycation and last, but not least, AGE-RAGE interaction leads to local inflammatory pathways [39]. One of the first experiments that studied these glycation products [40] proposed that the presence of AGEs excites macrophage cytokines,

which synthesize TNF-α (tumor necrosis factor) and IL-1. Crișan et al. studied histological and immunohistochemical aspects of UV-derived CML in sun-exposed and unexposed skin, in a group of 32 healthy patients subdivided into age categories [41]. Their findings indicated that AGE-CML had a high expression in both exposed and unexposed skin, while CML was 10% higher in exposed skin compared with unexposed skin, proportionally to age. Superficial dermal layers had higher levels of glycation products compared with the deeper layers. Starting from the hypothesis that obese people store more AGEs in tissues and fluids, skin AGE autofluorescence was assessed in Western European patients diagnosed with obesity, smokers or non-smokers, with or without other metabolic pathologies [42]. The researchers found a difference of almost 10% between obese and non-obese persons, which might be due to variations in the subjects' diet. Methylglyoxal was shown to affect collagen's extracellular matrix during aging, induces endoplasmic reticulum (ER) stress and apoptosis by activating the PERK-eIF2α and caspase-12 pathways, and by generating hydrogen peroxide and oxygen-derived free radicals [43]. A systematic review on AGEs influence in wound healing evidenced that AGEs reduce the elasticity and scar thickness, by disarranging collagen structure, producing short and thin fibers [44]. An *in vitro* multiscale mechanical testing using ribose and harvested human tendons samples showed that AGEs limit fiber-fiber and fibril-fibril sliding and by that the tissue viscoelasticity is strongly diminished [45]. A recent cross-sectional controlled study investigated the association between joint stiffness and AGEs in patients with type I diabetes. Anamnesis, clinical examination and skin biopsies- using liquid chromatography-mass spectrometry were performed. The results associated joint stiffness with long-term glycosylated hemoglobin and the AGEs MG and pentosidine [46]. Another study analyzed in rat tail and Achilles tendons fibrils mechanics and the effect of MG treatment [47]. Tendons from rats with the age of 4 and 16 weeks were used. The results showed no influence of the age on mechanical properties, but methylglyoxal generated stiffness without fragility of the tested tissues. Regarding hard structures, 170 human bone samples were investigated to evaluate the relationship between pentosidine and total AGEs in cortical and cancellous bone [48]. The result showed a higher pentosidine and total AGEs accumulation in cancellous bone compared to cortical bone. Thomas and co. evaluated CML in type I collagen bone samples, using mass spectrometry, and a protocol including demineralization, heating and trypsin digestion for the assessment of CML in bone tissue [49]. The study showed that CML is from 40 to 100 times higher in bone compared to pentosidine, which is correlated to the skeletal risk of fracture. The above accumulating evidence shows that skin autofluorescence could be correlated with the presence and severity of vascular complications of diabetes and could predict future cardiovascular events and death in patients with diabetes.

- **AGEs and metabolic disorders**

Mastrocola et al. gavaged mice with glucose 15% or fructose 15% during 30 weeks, with the purpose of assessing effects on weight, glucose tolerance and lipid profile [50]. The mice became overweight in a

proportion of up to 31%, glucose curves moved from the control at every glycemic bump, and the lipid profile was altered, with hepatic steatosis. GLAP and MOLD were overexpressed in the hepatic tissue of glucose-fed mice, while GOLD and CML were overexpressed in the hepatic tissue of fructose-fed mice. Moreover, the authors suggested the role of CML accumulation in hepatic steatosis, and their hypothesis is supported by other studies using *in vitro* and *in vivo* models [51,52]. In rat hepatocytes culture, the administration of ethanol led to severe downregulation and fatty accumulation in hepatocytes, because of oxidative stress induced by AA-AGEs [acetaldehyde (the main ethanol metabolite)-derived AGEs] [53]. AA-AGEs also led to apoptosis of neural cells, in a dose-dependent manner [54,55].

Worldwide, more than 10% of the population suffer from obesity, and it is estimated that by 2030, this proportion will double [56,57]. The link between AGEs accumulation and obesity development has been recently demonstrated in mice with genetically-induced deletion of leptin receptors, which were prone to consume excessive calories and develop obesity and insulin resistance. Obese mice showed high levels of CML trapped by the adipose tissue, while the deletion of RAGE reverted CML accumulation in adipose tissue, increasing the plasma levels; these findings indicated a RAGE-dependent mechanism underlying endogenous AGE-induced obesity [58]. A hypercaloric diet not only induces obesity, but also influences the cognitive function and memory [59,60]. A study conducted by Dahl et al. on showed that midlife patients with increased body mass index (BMI) had a decline in verbal and spatial skills [61].

- **AGEs and neurodegenerative diseases**

Several studies demonstrated the connection between dAGEs and neurodegenerative Alzheimer, Parkinson's disease and multiple sclerosis. Percentage differences of AGEs in the cerebrospinal fluid in Alzheimer's disease have been demonstrated [62]. An experiment focusing on the long-term effect of fructose consumption showed that fructose (10 times more damaging than sucrose) caused high serum AGEs levels and collagen alteration [63]. The authors suggested that AGEs affected the LDL (low density lipoprotein) entry into the brain mediated by astrocytes, and the consequent impairment of neural cell function [64]. Lysine represents a high percentage of apolipoproteins, a group of structural proteins in LDL, shown to be highly glycated. As a consequence, they become dysfunctional [65]. In inflammatory stress, the central nervous system reacts by gliosis, a non-specific hypertrophy of astrocytes, oligodendrocytes and microglia, viewed as protective feedback. Mastrocolla et al. evaluated the production of AGEs (marked as CML) in the hippocampal pyramidal cells of mice with a high-fructose diet [66]. The results expressed an increased amount of CML in pyramidal neurons, which activated the RAGE/NF-kB proinflammatory pathway, leading to general reactive gliosis.

- ***AGEs in carcinogenesis***

AGEs react with the $-NH_2$ group of nucleic proteins and could increase the risk of cancer. *In vitro* studies [67,42] showed that DNA incubation with glucose or a hyperglycemic medium induces mutagenic changes.

In addition to cigarette smoke, RAGE polymorphism is strongly involved in the development of oral squamous cell carcinoma (OSCC), lung and breast carcinoma [68,69]. High expression of RAGE in OSCC cell lines was associated with an increase in the depth and local recurrence of oral carcinomas [70,71]. HMGB1 can be synthesized intracellularly (as a physiologically stimulating growth factor), or extracellularly (with a proinflammatory effect). The association between HMGB1, released by RAGE, and NF-kB p65 increases melanoma inhibitory activity (MIA) in OSCC [72]. Moreover, S100A7 and S100A14, calcium-binding proteins, connect with RAGE receptors and promote epithelial-mesenchymal cell migration, cancer cell invasion and metastasis in osteosarcomas and cervical cancer [73,74].

- **AGEs in the saliva and their suitability as biomarker**

For the non-invasive diagnosis and monitoring of local and general diseases, saliva, as a biological fluid, is very accessible and easy to collect. Unlike blood collection, which is almost always uncomfortable and painful for the patient, or feces or urine analysis, which makes people feel embarrassed, saliva collection is highly accessible, by a simple spit into sterile recipients. Repeated samples can be collected, and diagnosis and monitoring using this method is an innovative and attractive approach.

Among saliva components, mucins are heavily glycosylated proteins, which are physiologically secreted by epithelial cells in the mouth. Their carbohydrate composition varies from tissue to tissue and is altered in response to mucosal infection and inflammation. Mucins in the oral cavity (including MUC5B, MUC7, MUC19, MUC1, and MUC4) protect the mucosa from pathogenic bacteria and noxious substances but provide favorable conditions to beneficial oral microorganisms through their carbohydrate receptors [75,76]. Salivary mucins are carriers for glycoproteins that have antibacterial activity; they transport these proteins, enable their retention on the surface of the teeth and prevent their degradation by forming complexes. The glycosylation of these proteins is also altered in response to environmental stress.

A study on healthy volunteers [77] assessed the variation of oxidative parameters in unstimulated saliva after mechanical removal of dental plaque, 30 days in a row. Salivary AGEs and AOPP (advanced oxidation protein products) were higher in men than in women, and there was a 60% variation between individuals. Salivary TBARS, markers of cell membrane peroxidation, and AOPP were analyzed mostly for their involvement in periodontal disease, as biomarkers of aggressiveness or efficacy of periodontal therapy [78-81]. Gingival attachment is an easily oxidized soft tissue, and markers accumulate in the adjacent saliva. The Comenius Research Unit in Bratislava [82] evaluated in a cross-sectional study of 82 pediatric patients salivary AGEs using unstimulated saliva, by spectrophotometric and spectrofluorometric measurements. The authors found differences in expression related to gender and oral hygiene, periodontal and dental status, but no differences related to age. This might be due to the physical condition not yet affected by metabolic diseases.

A particularity of the expression of AGEs is their increase in hyperglycemic conditions such as diabetes. This disease is strongly associated with a negative impact on soft and hard tissue tooth support, manifesting through

periodontitis. Salivary AGEs determinations using nuclear magnetic resonance spectra in a case-control study showed a strong association between the evolution of periodontal disease and AGEs accumulation, evidencing the implication of glycation products in oral diabetes complications [83]. AGEs spectrofluorometry was measured in the saliva, serum, urine and skin in 52 patients with type 2 diabetes [84]. AGEs accumulation in the skin was strongly age-related and changed in failure of renal function. Renal impairment might lead to the transfer of AGEs to other structures, such as skin.

Wautier et al. (2004) found that N-Carboxymethyl-lysine (CML-protein) blood levels were increased in patients with diabetes versus control group [85]. CML detection in saliva could represent an easy way of assessment of meta-inflammation in general diseases using a cavitas electrochemical sensor. Local infection found in oral cavity (such as periodontitis and other infection) must be excluded in order to have a correct interpretation of the results [86]. These non-invasive approaches of AGEs assessment in saliva for detection and monitoring general diseases, open new and future research areas for real time detection using wireless transmission.

- **AGEs in general diseases – Quo vadis?**

The results of *in vitro* and *in vivo* studies vary widely concerning the AGEs levels in biological fluids. These differences are influenced by many external and internal factors. The Western diet, vicious habits, age, sun overexposure increase and accelerate the production and deposition of external AGEs. The process is time and dose dependent, but there is no consensus as to when to start assessing AGEs levels. Should it be done in early childhood, when the body is theoretically free of deleterious substances, and should the obtained measurements be used as a gold standard, or in early adulthood in healthy patients? Systemic diseases, by altering immune host response, activation of proinflammatory mediators, create a favorable environment for the production of AGEs. Moreover, hyperglycemia, hyperlipidemic conditions such as diabetes, dyslipidemia and liver steatosis provide an abundant support for glycation product outcome, and thus, higher values/false positive values. This is why basic biochemical serum screening is important and should be evermore personalized. Urinary assessments might be influenced by an alteration of renal function, and AGEs might be redirected to other fluids or tissues. From the oral cavity, salivary AGEs might be collected using a cotton swab, in a routine dental examination, the results of which can offer valuable information. Oral cavity pathology could influence the values of salivary AGEs [87].

One of the reasons for which the assessment of AGEs is reliable is their chemical stability, once the Maillard reactions are completed. Even if a large amount is lost by urinary excretion, about 10% is identified in biological fluids and tissues. Animal and human studies have associated the amount of AGEs with physiological and pathological conditions. Among the tested sources, salivary samples are easy to collect and non-invasive, and create a comfortable alternative for the patient. However, protocols that involve providing a sterile environment, transport and examination create time and cost inconveniences.

- **AGEs downregulation strategies**

The source, body localization, ratio and last but not least, the presence of associated diseases has a great influence on the AGE treatment outcome. The current direction is to intervene in the glycation process at key points along its production mechanism, targeting specific pathways and receptors. One direction could be the use of antioxidant drugs. It has been reported that Ca^{2+} channel inhibitors, vitamins thiamine, P (flavonoids) and numerous natural compounds could help in the reduction of AGEs accumulation due to the antioxidant effect [88]. Experimental treatment with monoclonal anti-RAGE IgG3 resulted in improved glucose tolerance and attenuated renal complications in patients with type 2 diabetes [89]. The influence of local drug delivery such as chitosan-based pH-responsive hydrogels loaded with PTB in periodontopathic vs. non-periodontopathic groups was assessed by microcomputer tomography and histology. Results showed that PTB application could slow down the initiation of and improve recovery from experimental periodontitis [90]. It has been demonstrated that the association of antimicrobial doxycycline and ketone compounds such as flavonoids decreases AGE concentration in the crevicular fluid in periodontal disease [91]. Flavonoids inhibit ROS, NO, cyclooxygenase (COX), lipoxygenase and arachidonic acid pathways, which explains their role in reducing AGEs. One mechanism is the activation of dicarbonyl detoxification system, formed by glyoxalase I and II, enzymes which convert AGEs into hydroxyl acids [92]. The other mechanism is the increase of glyoxalase activity with lipoic acid, N-acetylcysteine, thiol-based antioxidants, and the subsequent downregulation of glycation product formation [93]. Trans-resveratrol and hesperetin polyphenols activate the glyoxalase system and lead to elimination of methylglyoxal (MG), property which is active both in normal body conditions- 36°-37° C and at high temperature during cooking [94]. Some dipeptide compounds- creatine and carnosine, have a carbonyl trapping effect, and thus reduce the formation and deposition of AGEs. Drinks such as red wine, green tea and various herbal extracts also have antiglycative effects [95]. Innovative approaches act on HMGB-1, WNT-1, S-100 RAGE (which have specificity in osteosarcoma) signaling pathways, and AGE-RAGE interactions. Guilbaud *et al.*(2016) suggested that a diet restriction in exogenous AGEs, but rich in purified nutrients, such as vitamins and natural antioxidants could limit the accumulation of glycation products [23]. It seems that diet restriction lowers early glycation products but not the advanced ones. Sell et al. measured furosine in skin collagen, and his results suggested that formation of CML and pentosidine depended not only on glycaemia, but also on oxidative stress and lipid oxidation [96]. A recent study showed that a diet which includes more than 100 grams of fructose daily is associated with weight increase among the patients [97]. Another study of Wang et al. showed that more than 200 grams/day fructose along with a hypercaloric diet led to increased uric acid serum levels [98]. Bunn and Higgins investigated the reactions between glucose, fructose and proteins. Their results showed that fructose is 7 times more reactive to produce Schiff bases, compared with glucose [99]. A meta-analysis study of Cozma *et al.* showed that the replacement of high glycemic index carbohydrates with fructose led to an improvement of glycemic index in diabetic patients, but more than 60 grams fructose /day led to serum

triglycerides increase, and a total cholesterol-lowering [100,101]. The above results state the negative influence of dietary intake in producing glycation compounds.

Due to their benefic effects, carnosine and creatine have been proposed as anti-AGEs strategies; creatine has the ability to block dicarbonyl compounds [102], and carnosine has antioxidant, anti-inflammatory and dicarbonyl-traping effects [103]. Deo et al. studied the effect of weight loss on CML and HbA1c serum levels, as glycation markers, in diabetic and non-diabetic patients. Their results showed 17% decrease in serum CML and reduction of HbA1c from 6.8% to 6.2% [104].

All these studies suggest that cautious food cooking (raw aliments, lower temperature and prolonged time), limited carbohydrate intake, and natural antioxidants supplements, could decrease the AGEs production.

- **Physical activity and DAGEs**

The first studies analyzing the effect of physical activity on glycation compounds were in Malaysian volunteers [105]. After 3 months of twice per week tai chi, serum AGEs and malondialdehyde decreased significantly. Another study analyzed methylglyoxal (MG) in erythrocytes after short and long run. The red cell MG decreased up to 60% in trained men vs 41% in untrained men [106]. A study which investigated endurance running with AGEs - pentosidine deposits in patellar tendons in 4 groups (elder master athletes vs. untrained elder with the average age of 64 years old and young endurance runners vs. untrained young men) found a 21% lower pentosidine density in athletes compared to untrained elderly [107]. Beside the results concerning AGEs, the thickness of the patellar tendons was higher in both elder and young trained men. The researchers suggested that long-term endurance training lowers the age-depending AGEs accumulation. A similar study compared circulating GO, MGO and 3-deoxyglucosone (3DG), serum CML, CEL, methylglyoxal-derived hydroimidazolone-1 (MG-H1), and pentosidine in elder endurance men vs. untrained men [108]. The results showed lower MGO, 3DG and MG-H1 concentration in elder athletes. An unexpected result was the concentration of CEL and CML, which were higher in athletes, and directly correlated with cardiorespiratory fitness. Macïas-Cervantes et al. experimented the effect of physical activity (3 times per week) on overweight men and reduced AGEs intake vs normal food intake for 3 months. AGEs decreased up to 50%, along with the increase of high-density lipoproteins (HDL) [109]. A prospective study on 98 volunteers investigated the effect of physical activity on circulating sRAGE (known to have an anti-inflammatory efficacy)- measured at baseline, 2, 6 and 8 months [110]. Increase of 9 to 22% of sRAGE were noticed after sport performing, more obvious in volunteers with initial low-performance. The above presented results suggest that physical training could lower AGEs depositing, and a long-term sport practice could have an antiglycative effect and a protective role in general diseases.

- **Conclusion**

Systemic diseases, by altering the immune host response and by activating the proinflammatory mediators, create a favorable environment for the production of AGEs. The Western diet, vicious habits, age, and sun overexposure increase and accelerate the production and deposition of AGEs, in a time and dose dependent manner. AGEs have a dose and time related cumulative negative effect on human tissues. Current anti-AGEs strategies include diet changes either modulation in food cooking, a physical active lifestyle, to increase antioxidative mechanism, and last but not least medication which interfere with the glycation process. Due to their stability in biological fluids, the validation of AGEs as biomarkers, including salivary AGEs, could represent a new approach in the early diagnosis and treatment of diet-related diseases, designing a long-lasting and efficient therapy.

- **Future Perspective:**

Due to their stability in biological fluids, the validation of AGEs as biomarkers in general diseases by means of accuracy would not be an impediment. In what concerns clinical results, summarized data show inhomogeneous AGE values in the tested samples. This might suggest that a greater number of subjects are required for *in vivo* study in order to achieve a statistically and clinically valid value of this metabolic biomarker.

Over time, salivary biosensors have been developed for several applications, including glucose evaluation, stress biomarkers and cortisol detection, salivary amylase and lactate activity, to name a few. However, to the best of our knowledge, there is no device produced and validated to measure salivary AGEs currently used in clinical settings, these were tested only for the research purposes. These biosensors could be attached to a mouth guard placed in the patient's oral cavity, and repeated non-invasive evaluations would be performed, for a real-time evaluation of AGEs modifications. Non-invasive monitoring of salivary AGEs could be used for the management of healthy subjects and particularly, patients affected by the general disease, and last but not least, for self-management at home. The non-invasive approaches of AGEs assessment in saliva open new and future research areas for real time detection using wireless transmission.

- **Executive Summary:**
- AGEs are represented by structures formed via glycation- a non-enzymatic reaction between sugars with a free amino or ketone group and proteins or lipids, and increase the food attractiveness for intake.
- AGEs can damage tissues by two different pathogenic pathways: receptor-mediated and receptor-independent manner. The current direction for therapies development is to intervene in the glycation process at key points along its production mechanism, targeting specific pathways and receptors. One direction could be the use of antioxidant drugs. Cautious in food cooking (raw aliments, lower temperature and prolonged time), limited carbohydrate intake, and natural antioxidants supplements, could decrease the AGEs production. Some results suggest that physical training could lower AGEs depositing, and a long-term sport practice could have an antiglycative effect and a protective role in general diseases.

- AGEs are considered to be a biomarker for aging, due to the continuous accumulation in body fluids and tissues: in collagen and elastin fibers and in the extracellular matrix, affecting its elasticity.
- A hypercaloric diet not only induces obesity, but also influences the cognitive function and memory. Several studies demonstrated the connection between dAGEs and neurodegenerative Alzheimer, Parkinson's disease and multiple sclerosis. In inflammatory stress, the central nervous system reacts by gliosis, a non-specific hypertrophy of astrocytes, oligodendrocytes and microglia, viewed as protective feedback.
- AGEs react with the -NH_2 group of nucleic proteins and could increase the risk of cancer. The cigarette smoke, RAGE polymorphism is strongly involved in the development of oral squamous cell carcinoma, lung and breast carcinoma.
- For the non-invasive diagnosis and monitoring of local and general diseases, saliva, as a biological fluid, is very accessible and easy to collect. Repeated samples can be collected, and diagnosis and monitoring using this method is an innovative and attractive approach. A salivary biosensor could be developed and attached to a mouth guard placed in the patient's oral cavity, and repeated non-invasive evaluations would be performed, for a real-time evaluation of AGEs modifications. Non-invasive monitoring of salivary AGEs could be used for the management of healthy subjects and patients affected by the general disease with possibility of self-management at home.

- **Figure legends** - the figures have incorporated the legend (symbols)

  Figure 1. Graphical abstract

  Figure 2. AGE sources, metabolic pathways and effects on storage organs

  Figure 3. The AGEs' negative effects on the human body

- **References:**

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Hodge I. Dehydrated Foods, Chemistry of Browning Reactions in Model Systems. *J Agric Food Chem*. 1, 928–943 (1953).
2. Neviere R, Yu Y, Wang L, et al. Implication of advanced glycation end products (Ages) and their receptor (Rage) on myocardial contractile and mitochondrial functions. *Glycoconj J*. 33(4), 607–617 (2016).
3. Aragno M, Mastrocola R. Dietary sugars and endogenous formation of advanced glycation endproducts: Emerging mechanisms of disease. *Nutrients.* 9(4), 1–16 (2017).
4. Uribarri J, Cai W, Sandu O, et al. Diet-derived advanced glycation end products are major contributors to the body's AGE pool and induce inflammation in healthy subjects. *Ann New York Acad Sci.* 1043, 461–466 (2005).
5. Brings S, Fleming T, Freichel M, et al. Dicarbonyls and advanced glycation end-products in the development of diabetic complications and targets for intervention. *Int j Mol Sci.* 18(5), pii:E984 (2017).
6. Goldberg T, Cai W, Peppa M, et al. Advanced glycoxidation end products in commonly consumed foods. *J Am Diet Assoc.* 104, 1287–1291 (2004).
7. Helou C, Gadonna-Widehem P, Robert N, et al. The impact of raw materials and baking conditions on Maillard reaction products, thiamine, folate, phytic acid and minerals in white bread. *Food Funct.* 7(6), 2498-2507 (2016).
8. Li L, Han L, Fu Q, et al. Formation and Inhibition of Nε-(Carboxymethyl)lysine in Saccharide-Lysine Model Systems during Microwave Heating. *Molecules.* 17(11), 12758–12770 (2012).
9. Fogliano V, Morales F. Estimation of dietary intake of melanoidins from coffee and bread. *Food & function.* 2(2), 117–123 (2011).
10. Tagliazucchi D, Bellesia A. The gastro-intestinal tract as the major site of biological action of dietary melanoidins.

Amino Acids. 47(6), 1077–1089 (2015).
11. Cerami C, Founds H, Nicholl I, et al. Tobacco smoke is a source of toxic reactive glycation products. *Proc Natl Acad Sci USA.* 9;94(25), 13915-13920 (1997).
12. Monnier V, Kohn R, Cerami A. Accelerated age-related browning of human collagen in diabetes mellitus. *Proc Natl Acad Sci USA.* 81, 583–587 (1984).
13. Tamanna N, Mahmood N. Food processing and maillard reaction products: Effect on human health and nutrition. *Int J Food Sci.* 2015:526762 (2015).
14. Poulsen MW, Andersen JM, Hedegaard RV, et al. Short-term effects of dietary advanced glycation end products in rats. *Brit J Nutr.* 115(04), 629–636 (2016).
15. Delgado-Andrade C, Fogliano V. Dietary Advanced Glycosylation End-Products (dAGEs) and Melanoidins Formed through the Maillard Reaction: Physiological Consequences of their Intake. *Annu Rev Food Sci Technol.* 25(9), 271–291 (2018).
16. Scheijen J, Hanssen NMJ, van Greevenbroek MM, et al. Dietary intake of advanced glycation endproducts is associated with higher levels of advanced glycation endproducts in plasma and urine: The CODAM study. *Clin Nutr.* 37(3), 919-925 (2018).
17. Birlouez-Aragon I, Saavedra G, Tessier FJ, et al. A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. *Am J Clin Nutri.* 91, 1220–1226 (2010).
18. Li M, Zeng M, He Z, et al. Increased accumulation of protein-bound N (carboxymethyl)lysine in tissues of healthy rats after chronic oral n (carboxymethyl)lysine. *J Agric Food Chem.* 63(5), 1658–1663 (2015).
19. Vlassara H, Cai W, Crandall J, et al. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc Nat Acad Sci USA.* 99(24), 15596–601 (2002).
20. Uribarri J, Peppa M, Cai W, et al. Restriction of dietary glycotoxins reduces excessive advanced glycation end products in renal failure patients. *J Am Soc Nephrol.* 14(3), 728–731 (2003).
21. Förster A, Kühne Y, Henle T. Studies on absorption and elimination of dietary Maillard reaction products. *Annals of the New York Academy of Sciences.* 1043, 474–481 (2005).
22. Alamir I, Niquet-Leridon C, Jacolot P, et al. Digestibility of extruded proteins and metabolic transit of N ε -carboxymethyllysine in rats. *Amino Acids.* 44(6), 1441–1449 (2013).
23. Guilbaud A, Niquet-Leridon C, Boulanger E, et al. How Can Diet Affect the Accumulation of Advanced Glycation End-Products in the Human Body? *Foods.* 5(4), 84 (2016).
24. Cefalu WT, Bell-Farrow AD, Wang ZQ, et al. Caloric restriction decreases age-dependent accumulation of the glycoxidation products, N"-(carboxymethyl)lysine and pentosidine, in rat skin collagen. *J Gerontol A Biol Sci Med Sci.* 50, B337–B341 (1995).
25. Morales F, Somoza V, Fogliano V. Physiological relevance of dietary melanoidins. *Amino Acids.* 42(4), 1097–109 (2012).
26. Finot PA, Magnenat E. Metabolic transit of early and advanced Maillard products. *Prog Food Nutr Sci.* 5(1–6), 193–207 (1981).
27. Koschinsky T, He C, Mitsuhashi T, et al. Orally absorbed reactive glycation products (glycotoxins): An environmental risk factor in diabetic nephropathy. *Proc Natl Acad Sci USA.* 94(12), 6474–6479 (1997).
28. O'Brien JD, Thompson DG, Day SJ, et al. Perturbation of upper gastrointestinal transit and antroduodenal motility by experimentally applied stress: the role of beta-adrenoreceptor mediated pathways. *Gut.* 30(11), 1530-1539 (1989).
29. Finot PA, Magnenat E. Le blockage de la lysine par la reaction de Maillard. II. Propriete chimiques des derives N-(desoxy-1-D-fructosyl-1) et N-(dCsoxy-l-D-lactulosyl1-1) de la lysine. *Helv Chim Acta.* 55, 1153–1164 (1972).
30. Erbersdobler HF. Determination of lysine damage and calculation of lysine bio-availability in several processed foods. *Z Ernahrungswiss.* 30(1), 46–94 (1991).
31. Mehta BM, Deeth HC. Blocked Lysine in Dairy Products: Formation, Occurrence, Analysis, and Nutritional Implications. *Comprehensive Reviews in Food Science and Food Safety.* 15(1), 206–218 (2016).
32. Uribarri J, Woodruff S, Goodman S, et al. Advanced glycation end products in foods and a practical guide to their reduction in the diet. *J Am Diet Assoc.* 110(6), 911–916 (2010).
33. Hull GLJ, Woodside J, Ames J, et al. N-epsilon-(carboxymethyl)lysine content of foods commonly consumed in a western style diet. *Food Chem.* 131(1), 170–174 (2012).

34. Scheijen J, Clevers E, Engelen L, et al. Analysis of advanced glycation endproducts in selected food items by ultra-performance liquid chromatography tandem mass spectrometry: presentation of a dietary AGE database. *Food Chem.* 190, 1145–1150 (2016).
35. Neeper M, Schmidt AM, Brett J, et al. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem.* 25(267(21)), 14998–15004 (1992).
36. Uribarri J, del Castillo MD, de la Maza MP, et al. Dietary advanced glycation end products and their role in health and disease. *Adv Nutr.* 6, 461–473 (2015).
37. Semba RD, Gebauer SK, Baer DJ, et al. Dietary Intake of Advanced Glycation End Products Did Not Affect Endothelial Function and Inflammation in Healthy Adults in a Randomized Controlled Trial. *J Nutr.* 7, 1037–1042 (2014).
38. Bastos D, Gugliucci A. Contemporary and controversial aspects of the Maillard reaction products. *Curr Opin Food Sci.* 1, 13–20 (2015).
39. Kim CS, Park S, Kim J. The role of glycation in the pathogenesis of aging and its prevention through herbal products and physical exercise. *J Exerc Nutrition Biochem*. 21(3), 55–61 (2017).
40. Vlassara H, Brownlee M, Manogue K, et al. Cachectin/TNF and IL-1 induced by glucose-modified proteins: role in normal tissue remodeling. *Science.* 240(4858), 1546–1548 (1988).
41. Crisan M, Taulescu M, Crisan D, et al. Expression of Advanced Glycation End-Products on Sun-Exposed and Non-Exposed Cutaneous Sites during the Ageing Process in Humans. *PLOS ONE.* 8(10), e75003 (2013).
42. den Engelsen C, van den Donk M, Gorter KJ, et al. Advanced glycation end products measured by skin autofluorescence in a population with central obesity. *Dermatoendocrinol.* 4(1), 33–38 (2012).
43. Nowotny K, Castro JP, Hugo M, et al. Oxidants produced by methylglyoxal-modified collagen trigger ER stress and apoptosis in skin fibroblasts. *Free Rad Biol Med.* 120, 102–113 (2018).
44. Van Putte L, De Schrijver S, Moortgat P. The effects of advanced glycation end products (AGEs) on dermal wound healing and scar formation: a systematic review. *Scars, Burns & Healing.* 2, 1-14 (2016).
45. Gautieri A, Passini FS, Silván U, et al. Advanced glycation end-products: Mechanics of aged collagen from molecule to tissue. *Matrix Biol.* 59, 95–108 (2017).
• T**his study suggests that AGEs determine functional and biological effects in collagen connective tissues through decrease tissue viscoelasticity (limiting fiber-fiber and fibril-fibril sliding).**
46. Holte KB, Juel NG, Brox JI, et al. Hand, shoulder and back stiffness in long-term type 1 diabetes; cross-sectional association with skin collagen advanced glycation end-products. The Dialong study. *J Diabetes Complications.* 31(9), 1408–1414 (2017).
47. Svensson RB, Smith ST, Moyer, PJ, et al. Effects of maturation and advanced glycation on tensile mechanics of collagen fibrils from rat tail and Achilles tendons. *Acta Biomaterialia.* 70, 270–280 (2018).
48. Karim L, Tang SY, Sroga GE, et al. Differences in non-enzymatic glycation and collagen cross-links between human cortical and cancellous bone. Osteoporosis Int. 24(9), 2441–2447 (2013).
49. Thomas CJ, Cleland TP, Sroga GE, et al. Accumulation of carboxymethyl-lysine (CML) in human cortical bone. *Bone.* 110, 128–133 (2018).
50. Mastrocola R, Collino M, Rogazzo M, et al. Advanced glycation end products promote hepatosteatosis by interfering with SCAP-SREBP pathway in fructose-drinking mice. Am J Physiol Gastrointest Liver Physiol. 305: G398–G407 (2013).
51. Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-sweetened, not glucose sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest.* 119, 1322–1334 (2009).
52. Gaens KH, Niessen PM, Rensen SS, et al. Endogenous formation of Nε-(carboxymethyl) lysine is increased in fatty livers and induces inflammatory markers in an in vitro model of hepatic steatosis. *J Hepatol.* 56, 647–655 (2012).
• **Nε-(carboxymethyl) lysine accumulation is significantly associated with the grade of hepatic steatosis and hepatic inflammation, and also with levels of inflammatory markers (their gene expression).**
53. Hayashi N, George J, Takeuchi M, et al. Acetaldehyde-Derived Advanced Glycation End-Products Promote Alcoholic Liver Disease. *PLOS ONE*. 8(7), e70034 (2013).
54. Takeuchi M, Watai T, Sasaki N, et al. Neurotoxicity of acetaldehyde-derived advanced glycation end products for cultured cortical neurons. *J Neuropathol Exp Neurol.* 62, 486–496 (2003).

55. Takeuchi M, Saito T. Cytotoxicity of acetaldehyde-derived advanced glycation end-products (AA-AGE) in alcoholic-induced neuronal degeneration. *Alcohol Clin Exp Res.* 29(12): 220S–224S (2005).
56. Wild S, Roglic G, Green A, et al. Global prevalence of diabetes. *Diabetes Care.* 27, 1047–1053 (2004).
57. Danaei G, Finucane MM, Lu Y, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet.* 378, 31–40 (2011).
58. Gaens KH, Goossens GH, Niessen PM, et al. N-(carboxymethyl)lysine-receptor for advanced glycation end product axis is a key modulator of obesity-induced dysregulation of adipokine expression and insulin resistance. *Arterioscler Thromb Vasc Biol.* 34(6), 1199–1208 (2014).
59. Lakhan SE, Kirchgessner A. The emerging role of dietary fructose in obesity and cognitive decline. Nutr J. 12, 114. (2013).
60. Hsu TM, Konanur VR, Taing L, et al. Effects of Sucrose and High Fructose Corn Syrup Consumption on Spatial Memory Function and Hippocampal Neuroinflammation in Adolescent Rats. *HIPPOCAMPUS.* 25, 227–239 (2015).
61. Dahl A, Hassing LB, Fransson E, et al. Being overweight in midlife is associated with lower cognitive ability and steeper cognitive decline in late life. *J Gerontol A Biol Sci Med Sci.* 65, 57–62 (2010).
62. Shuvaev VV, Laffont I, Serot JM, et al. Increased protein glycation in cerebrospinal fluid of Alzheimer's disease. *Neurobiol Aging.* 22, 397–402 (2001).
63. Levi B, Werman MJ. Long-term fructose consumption accelerates glycation and several age-related variables in male rats. *J Nutr.* 128, 1442–1449 (1998).
64. Seneff S, Wainwright GML. Nutrition and Alzheimer's disease: The detrimental role of a high carbohydrate diet. *Eur J Int Med.* 22: 134–140 (2011).
65. Younis N, Sharma R, Soran H, et al. Glycation as an atherogenic modification of LDL. *Curr Opin Lipidol.* 19, 378–384 (2008).
66. Mastrocola R, Nigro D, Cento AS, et al. High-fructose intake as risk factor for neurodegeneration: Key role for carboxy methyllysine accumulation in mice hippocampal neurons. *Neurobiol Dis.* 89: 65–75 (2016).
•• **Carboxy methyllysine accumulation in hippocampal neurons triggered by high-fructose diet determine the same molecular and metabolic alterations observed in early phases of neurodegenerative diseases, and can represent a risk factor for their onset.**
67. Lee A, Cerami A. Elevated glucose 6-phosphate levels are associated with plasmid mutations in vivo. *Proc Natl Acad Sci USA.* 84, 8311–8314 (1987).
68. Brownlee M. Negative consequences of glycation. *Metabolism.* 49(2), S1: 9-13 (2000).
69. Su M, Chien M, Lin C. RAGE gene polymorphism and environmental factor in the risk of oral cancer. *J Dent Res*. 94(3), 403–411 (2015).
70. Bhawal UK, Ozaki Y, Nishimura M, et al. Association of expression of receptor for advanced glycation end products and invasive activity of oral squamous cell carcinoma. *Oncology.* 69(3), 246–255 (2005).
71. Huang ZH, Zhao DC, Lu HW. Association between the receptor for advanced glycation end products gene polymorphisms and cancer risk: a systematic review and meta-analysis. *J BUON.* 20(2), 614–624 (2015).
72. Sasahira T, Kirita T, Bhawal UK, et al. Receptor for advanced glycation end products (RAGE) is important in the prediction of recurrence in human oral squamous cell carcinoma. *Histopathology.* 51(2), 166–172 (2007).
73. Sasahira T, Kirita T, Oue N, et al. High mobility group box-1-inducible melanoma inhibitory activity is associated with nodal metastasis and lymphangiogenesis in oral squamous cell carcinoma. *Cancer Sci.* 99(9), 1806–1812 (2008).
74. Kataoka K, Ono T, Murata H, et al. S100A7 promotes the migration and invasion of osteosarcoma cells via the receptor for advanced glycation end products. *Oncol Lett.* 3(5), 1149–1153 (2012).
75. Gibbins HL, Proctor GB, Yakubov GE, et al. Concentration of salivary protective proteins within the bound oral mucosal pellicle. *Oral Dis.* 20(7), 707–713 (2014).
76. Frenkel ES, Ribbeck K. Salivary mucins protect surfaces from colonization by cariogenic bacteria. *AEM.* 81(1), 332–338 (2015).
77. Lettrichová I, Morvová M, Šikurova L. Saliva as diagnostic fluid: Its applicability to noninvasive evaluation of oxidative stress status and ovulation detection. Bratislava: Comenius University, Mlynská dolina 842 48, Bratislava. (2015).
78. Sculley D, Langley-Evans S. Periodontal disease is associated with lower antioxidant capacity in whole saliva and

evidence of increased protein oxidation. *Clin Sci.* 105, 167–172 (2003).
79. Guentsch A, Preshaw P, Bremer-Streck S, et al. Lipid peroxidation and antioxidant activity in saliva of periodontitis patients: effect of smoking and periodontal treatment. *Clin Oral Invest.* 12, 345–352 (2008).
80. Behuliak M, Pálffy R, Gardlík R, et al. Variability of thiobarbituric acid reacting substances in saliva. *Dis Markers.* 26, 49–53 (2009).
81. Wei D, Zhang XL, Wanag Y, Lipid peroxidation levels, total oxidant status and superoxide dismutase in serum, saliva and gingival crevicular fluid in chronic periodontitis patients before and after periodontal therapy. *Aust Den J.* 55, 70–78 (2010).
82. Tothova' L, Celecov' V, Celeca P. Salivary markers of oxidative stress and their relation to periodontal and dental status in children. *Dis Markers.* 34, 9–15 (2013).
83. Yoon MS, Jankowski V, Montag S, et al. Characterisation of advanced glycation end products in saliva from patients with diabetes mellitus. *Biochem Biophys Res Commun.* 323(2), 377–381 (2004).
84. Garay-Sevilla ME, Regalado JC, Malacara JM, et al. Advanced glycosylation end products in skin, serum, saliva and urine and its association with complications of patients with Type 2 diabetes mellitus. *J Endocrinol Invest.* 28(5), 223–230 (2005).
• **The AGEs assessment in skin, serum and saliva can be correlated with diabetes complications.**
85. Wautier MP, Boulanger E, Guillausseau PJ, et al. AGEs, macrophage colony stimulating factor and vascular adhesion molecule blood levels are increased in patients with diabetic microangiopathy. *Thromb Haemost.* 91(5), 879-885 (2004).
86. Ciui B, Tertis M, Feurdean CN, et al. Cavitas electrochemical sensor toward detection of N-epsilon (Carboxymethyl)lysine in oral cavity. *Sensors & Actuators: B. Chemical.* 281, 399–407 (2019). In press, doi.org/10.1016/j.snb.2018.10.096
•• **The first cavitas printed electrochemical sensor for the direct salivary detection of N€(Carboxymethyl)lysine integrated in a mouth guard.**
87. Ilea A, Băbţan AM, Boşca AB, et al. Advanced glycation end products (AGEs) in oral pathology. *Arch Oral Biol.* 18;93, 22-30 (2018)
88. Sadowska-Bartosz I, Bartosz G. Prevention of Protein Glycation by Natural Compounds. *Molecules.* 20(2), 3309–3334 (2015).
89. Grauballe MB, Østergaard JA, Schou S, et al. Blockade of RAGE in Zucker obese rats with experimental periodontitis. *J Periodontal Res.* 52(1), 97–106 (2017).
90. Yu MC, Chang CY, Chao YC, et al. Ph-Responsive Hydrogel With an Anti-Glycation Agent for Modulating Experimental Periodontitis. *J Periodontol.* 87, 742–748 (2016).
91. Engebretson SP, Hey-Hadavi J. Sub-antimicrobial doxycycline for periodontitis reduces hemoglobin A1c in subjects with type 2 diabetes: a pilot study. *Pharmacol Res.* 64(6), 624–629 (2011).
92. Thornalley P. The glyoxalase system in health and disease. *Mol Aspects Med.* 14, 287–371 (1993).
93. Suh JH, Wang H, Liu RM, et al. (R)-alpha-lipoic acid reverses the age-related loss in GSH redox status in post-mitotic tissues: evidence for increased cysteine requirement for GSH synthesis. *Arch Biochem Biophys.* 1;423(1), 126-135 (2004).
94. Lo CY, Li S, Tan D, et al. Trapping reactions of reactive carbonyl species with tea polyphenols in simulated physiological conditions. *Mol Nutr Food Res.* 50, 1118–1120 (2006).
95. Vlassopoulos A, Lean MEJ, Combet E. Oxidative stress, protein glycation and nutrition - interactions relevant to health and disease throughout the lifecycle. *Proc Nutr Soc.* 73(3), 430–438. (2014).
96. Sell DR, Lane MA, Obrenovich ME, et al. The effect of caloric restriction on glycation and glycoxidation in skin collagen of nonhuman primates. *J Gerontol A Biol Sci Med Sci.* 95, 508–516 (2003).
97. Sievenpiper JL, de Souza RJ, Mirrahimi A, et al. Effect of fructose on body weight in controlled feeding trials: A systematic review and meta-analysis. *Ann Intern Med.* 156, 291–304 (2012).
98. Wang DD, Sievenpiper JL, de Souza RJ, et al. The effects of fructose intake on serum uric acid vary among controlled dietary trials. *J Nutr.* 142(5), 916–923 (2012).
99. Bunn HF, Higgins PJ. Reaction of monosaccharides with proteins: Possible evolutionary significance. *Science.* 213, 222–224 (1981).
100. Cozma AI, Sievenpiper JL, de Souza RJ, et al. Effect of Fructose on Glycemic Control in Diabetes. *Diabetes Care.*

35, 1611–1620 (2012).
101. Sievenpiper JL, Carleton AJ, Chatha S, et al. Heterogeneous effects of fructose on blood lipids in individuals with type 2 diabetes: systematic review and meta-analysis of experimental trials in humans. *Diabetes Care.* 32, 1930–1937 (2009).
102. Lobner J, Degen J, Henle T. Creatine is a scavenger for methylglyoxal under physiological conditions via formation of N-(4-methyl-5-oxo-1-imidazolin-2-yl)sarcosine (MG-HCr). *J Agric Food Chem.* 63, 2249–2256 (2015).
103. Javadi S, Yousefi R, Hosseinkhani S, et al. Protective effects of carnosine on dehydroascorbate-induced structural alteration and opacity of lens crystallins: Important implications of carnosine pleiotropic functions to combat cataractogenesis. *J Biomol Struct Dyn.* 19, 1–19 (2016).
104. Deo P, Keogh JB, Price NJ, et al. Effects of weight loss on advanced glycation end products in subjects with and without diabetes: A preliminary report. *Int J Environ Res Public Health.* 14(12), 1–8 (2017).
105. Goon JA, Noor AH, Musalmah M, et al. Effect of tai chi exercise on DNA damage, antioxidant enzymes, and oxidative stress in middle-age adults. *J Phys Act Health.* 6, 43–54 (2009).
106. Kondoh Y, Kawase M, Ohmori S. D-lactate concentrations in blood, urine and sweat before and after exercise. *Eur J Appl Physiol Occup Physiol.* 65, 88–93 (1992).
107. Couppé C, Svensson RB, Grosset JF, et al. Life-long endurance running is associated with reduced glycation and mechanical stress in connective tissue. *Age.* 36(4), 9655 (2014).
108. Maessen MFH, Schalkwijk CG, Verheggen RJHM, et al. A comparison of dicarbonyl stress and advanced glycation endproducts in lifelong endurance athletes vs. sedentary controls. *J Sci Med Sport.* 20(10), 921–926 (2017).
109. Macías-Cervantes MH, Rodríguez-Soto JMD, Uribarri J, et al. Effect of an advanced glycation end product-restricted diet and exercise on metabolic parameters in adult overweight men. *Nutrition.* 31(3), 446–451 (2015).
110. Sponder M, Campean IA, Emich M, et al. Long-term physical activity leads to a significant increase in serum sRAGE levels: a sign of decreased AGE-mediated inflammation due to physical activity? *Heart and Vessels.* 33(8), 893–900 (2018).