Advanced glycation end products (AGEs): Emerging Mediators of Skin Aging

Paraskevi Gkogkolou and Markus Böhm

Abstract
Advanced glycation end products (AGEs) derive from nonenzymatic reactions between reducing sugars and proteins, lipids or nucleic acids. In this chapter, we highlight the role of AGEs as an emerging class of mediators of skin aging. After a short section on the biochemistry and biology of AGEs we will put these molecules into the context of skin aging. Evidence will be provided that: (1) AGEs are detectable in skin, (2) that they accumulate over time in aged skin, and (3) that they act via diverse mechanisms (receptor and nonreceptor-mediated) on various cellular and noncellular targets of the skin. Special emphasis will be devoted to the connections between AGEs and reactive oxygen species, the latter established players of cutaneous aging. Finally, current and future strategies are described by which the impact of AGEs on skin aging may be counteracted.

Introduction
Advanced glycation end products (AGEs) derive from nonenzymatic reactions between reducing sugars, such as glucose and proteins, lipids or nucleic acids. This process is called glycation [1] and is distinguished from glycosylation, which is an enzymatic procedure. Glycation was first described by Maillard in 1912 [2] but its role in food browning during thermal processing was disclosed by Hodge only 50 years later [3].
Since then, AGEs have been detected in various tissues during aging but especially in patients with diabetes where elevated glucose levels exist. Moreover, deposition of AGEs has been implicated in a number of diabetes- and age-associated complications such as diabetic angiopathy [4], neurodegenerative disorders, and osteoarthritis [5].

In the last years, the potential role of AGEs in skin aging has attracted many scientists. Targeting AGE-mediated pathways could become a novel strategy to prevent not only diabetes-related complications but also to promote healthier aging in general and to prevent aging of the skin.

**A Brief Biochemistry of AGEs**

AGEs are nonenzymatic reaction products between reactive sugars such as glucose and various other molecules. During the formation of AGEs simple and complicated multistep reactions are involved. During the classical Maillard reaction, strong, reactive, electrophilic carbonyl groups of glucose or other sugars (fructose, ribose) react with free amine groups of neutral amino acids (usually lysine or arginine), nucleic acids, or lipids, leading to formation of a nonstable Schiff base [1]. In a second rearrangement reaction, a more stable ketoamine (Amadori product) is formed. Schiff bases and Amadori products are reversible reaction products. These can further irreversibly react with amino acid residues of peptides and proteins leading to formation of stable protein adducts or protein crosslinks [1]. Various oxidation, dehydration, polymerization, and oxidative breakdown reactions can give rise to numerous other AGEs. Oxygen, reactive oxygen species (ROS), and redox active transition metals accelerate these nonenzymatic reactions. If an oxidation process is involved, these products are called advanced glycoxidation end products.

Biochemically, AGEs are a heterogeneous group of molecules. Since discovery of the first glycated protein, glycated hemoglobin in diabetes patients, numerous other AGEs have been identified. Some of them have characteristic autofluorescent properties, which facilitates their identification in situ after tissue sampling but may also allow in vivo detection by noninvasive methods. Table 1 highlights the most commonly found AGEs in the skin.

*N*-(*Carboxymethyl)lysine (CML) is the most common AGE in vivo and the major epitope of

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*Abbreviations: ELISA enzyme-linked immunosorbent assay, GO glyoxal, HPLC high performance liquid chromatography, IHC immunohistochemistry, IB immunoblotting, IF immunofluorescence, LC-ESI-TOF-MS liquid chromatography–electrospray ionization time-of-flight mass spectrometry, LC/MS liquid chromatography/mass spectrometry, MGO methylglyoxal, SIM/GC-MS selected ion monitoring gas chromatography-mass spectrometry, SC stratum corneum, SG stratum granulosum, SS stratum spinosum; all other abbreviations are already explained in the text.*
many commercially available anti-AGE antibodies [13]. It is a nonfluorescent protein adduct and formed via oxidative degradation of Amadori products or direct addition of glyoxal to lysine.

Pentosidine is composed of an arginine and a lysine residue crosslinked to a pentose [14]. It is a fluorescent glycoxidation product and represents the major AGE involved in protein-protein crosslinks.

3-Deoxyglucosome (3GO), methylglyoxal (MG), and glyoxal (GO) are very reactive dicarbonyl compounds which mainly derive from oxidative degradation or autoxidation of Amadori products and typically lead to molecular crosslinking [1, 15].

Other in vivo detected AGEs include glucosepane, carboxymethyl-hydroxy-lysine, carboxyethyllysine (CEL), fructoselysine, methylglyoxal-derived hydroimidazolones, and pyrraline. They form nonfluorescent protein adducts while glyoxal-lysine dimer (GOLD) and methylglyoxal-lysine dimer (MOLD) form nonfluorescent protein crosslinks [1].

AGEs are generated at low rates as endogenous by-products during normal metabolic processes. As expected, increased bioavailability of glucose as found in diabetes but also smoking and ultraviolet (UV) irradiation increase formation of AGEs [9, 16]. AGEs may be also exogenously inserted into the organism via diet, with approximately 10–30 % of ingested AGEs reaching the circulation [17]. The content of AGEs in food depends on the method of preparation, i.e., especially cooking and temperature, with fried food containing higher amounts of AGEs than boiled or steamed food [18]. Finally, it seems that the level of circulating AGEs is genetically determined [19].

Once formed, AGEs can be removed from the organism via intrinsic detoxifying mechanisms. Two isoforms of glyoxalase (Glo) utilize reduced glutathione to catalyze the conversion of glyoxal, methylglyoxal, and other α-oxoaldehydes to the less toxic D-lactate [20]. The intracellular fructosamine kinases phosphorylate and destabilize Amadori products leading to their spontaneous breakdown [21]. Fructosamine-3-kinase (FN3K), one of the most studied enzymes in this system, is almost ubiquitously expressed in human tissues including the skin and plays an important role on the intracellular breakdown of Amadori products [21]. The fructosyl-amino oxidases (FAQOs) or “amadoriases” also recognize and oxidatively break Amadori products; however, these are expressed in bacteria, yeast, and fungi but not in mammals [21]. Finally, cathepsins D and L are capable of degrading endocytosed AGE-modified proteins [22].

Receptors of AGEs

Various receptors and binding proteins for AGEs have been identified. The most thoroughly investigated one is RAGE (receptor for AGEs). It belongs to the immunoglobulin superfamily of cell surface receptors and is encoded by a gene on chromosome 6 near the major histocompatibility complex III. RAGE is a pattern recognition receptor and binds in addition to AGEs other molecules such as S-100/calgranulins, high motility group protein B1 (amphoterine), amyloid β-peptides, and beta-sheet fibrils [23]. Mitogen-activated protein kinases (MAPKs), extracellular signal-related kinases (ERK) 1 and 2, p38, stress-activated protein kinase-c/Jun-N-terminal kinase (SAPK/JNK), phosphatidylinositol 3 kinase (SAPK/JNK), phosphatidylinositol 3 kinase (PI-3K), the janus kinases, and protein kinase C are activated in a cell type-specific manner upon engagement of RAGE with a ligand. RAGE stimulation leads to sustained and self-perpetuating activation of NF-κB and transcription of many proinflammatory genes like tumor necrosis factor-α, interleukin (IL)-6, and C-reactive protein (CRP). Furthermore, RAGE activation directly increases oxidative stress via activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, inactivation of antioxidant enzymes like superoxide dismutase (SOD) and catalase, as well as via reduction of glutathione (GSH), the latter a coenzyme of the major AGE-degrading enzyme Glo I [24].

In addition to the cell-bound RAGE, soluble RAGE (sRAGE) and endogenous secretory RAGE have been identified as binding partners for AGEs. Both are extracellular truncated forms of RAGE containing the ligand-binding domain but not the transmembrane domain. They are
formed via alternative gene splicing and post-translational proteolysis of RAGE. Due to the lack of the transmembrane domain they do not elicit signaling and are considered as decoy receptors of AGEs which counteract RAGE-mediated signaling [25].

Another group of AGE-receptors include macrophage scavenger receptor types I and II, oligosaccharyl transferase-48 (AGE-R1), 80K-H phosphoprotein (AGE-R2), and galectin-3 (AGE-R3) which are thought to regulate endocytosis and degradation of AGEs and counteract the effects of RAGE. AGE-R1 has been further shown to counteract AGE-induced oxidative stress via inhibition of RAGE signaling [26].

RAGEs are almost ubiquitously expressed in the organism, typically in low numbers. Expression of RAGE is upregulated in response to pathologic conditions [23, 27]. In skin, RAGE is expressed within epidermis and dermis, and its expression is higher in sun-exposed sites. A wide variety of human cell types including keratinocytes, fibroblasts, endothelial cells, and immune cells (dendritic cells, monocytes) express RAGE in vitro and in vivo.

Detection of AGEs in Aged Skin

Skin, due to its direct accessibility, offers excellent opportunities for direct detection of glycation using minimal invasive techniques in skin biopsies or even noninvasive techniques which take advantage of the autofluorescent properties of AGEs. Initially, AGEs deposition in the skin was studied with Western blots or autofluorescence in skin biopsies. Recently, AGE-Readers (DiagnOptics™ B.V., Groningen, The Netherlands; TruAge Scanner™ Morinda, Orem, UT, USA) were introduced in the market as a noninvasive method for in vivo measurements of AGEs accumulation based on their characteristic autofluorescence [28].

Skin glycation has been thoroughly investigated not only in diabetic skin but also in intrinsically and extrinsically aged skin. Glycation-associated autofluorescence correlated with chronological aging in a large number of nondiabetic subjects [29]. Moreover, AGEs are more abundant in sun-exposed skin in sites of solar elastosis [7]. Smoking has been shown to enhance formation of AGEs and increase their deposition in various tissues including skin [9]. Although a correlation of dietary AGEs with serum levels of AGEs has been shown, a possible correlation with skin AGEs has not been investigated yet.

Since most AGEs are formed via slow, nonenzymatic reactions it was initially believed that their accumulation depends on protein turnover rate, with long-lived proteins like collagen as well as elastin and fibronectin being the major targets of glycation [8]. Accordingly, presence of glycated collagen is first observed at the age of 20, with a yearly accumulation rate of about 3.7 % and reaching a 30–50 % increase at 80 years of age [7, 30]. However, CML was recently identified in keratin 10 of the upper epidermal layers of healthy subjects, suggesting a potential involvement of short-lived proteins in glycation [6]. In accordance with this, both epidermis and dermis were modified by glycation in an in vitro reconstructed skin organ model [31].

Interestingly, skin glycation has been shown to correlate with various systemic diabetes- and age-related complications such as angiopathy and chronic renal disease [32].

These findings indicate that skin glycation is not only a marker for diabetic monitoring but also – at least in nondiabetic individuals – a read-out for the extent of skin aging.

Cellular and Noncellular Targets of AGEs Within the Skin

Advanced glycation can directly act on a variety of biomolecules including proteins, lipids, and nucleic acids in the intracellular and extracellular compartments. These modifications can alter enzyme-substrate interactions, protein-DNA interactions, and protein-protein interactions, affecting numerous physiological functions of the organism. Moreover, AGEs react with RAGE to elicit genomic and nongenomic effects that modulate cell homeostasis, metabolism, redox balance, matrix protein turnover, and immune and inflammatory responses.
Extracellular Matrix Proteins

Extracellular matrix (ECM) proteins not only create a supportive framework for skin cells but also directly interact with them regulating important cellular functions such as migration, differentiation, and proliferation. Due to their long lifetime before degradation they represent the molecules which mainly suffer from glycation.

Crosslinking of adjacent collagen results in increased stiffness and decreased resistance to mechanical forces [33]. AGE-mediated changes on side chains of collagen affect its contact sites with cells and other matrix proteins and consequently its ability to react with them [34]. For example, actin polymerization and migration of immune cells is subsequently impaired [35]. The precise aggregation of monomers into the triple helix and the association of collagen IV with laminin in the basal membrane are affected. Moreover, degradation by matrix metalloproteinases (MMPs) is impaired, thus inhibiting removal of modified collagen and replacement by newly synthesized and functional one [5]. Accordingly, tissue permeability and turnover is impaired.

Other ECM proteins suffering from advanced glycation are elastin [7, 9] and fibronectin [8]. CML-modified elastin assembles in large and irregular structures, has decreased elasticity, and is resistant to proteolytic degradation [36]. CML-modified elastin is found almost exclusively in sites of actinic elastosis and not in sun-protected skin. UV irradiation stimulates glycation of elastin in the presence of sugars in vitro. Glycation of fibronectin impairs its interaction with integrin αvβ1 and delays wound healing [37].

In vitro glycated skin samples have impaired biomechanical properties [38]. In vivo, decreased skin elasticity characterizes diabetic subjects in comparison to healthy controls [39].

Intracellular Proteins

AGEs modify and lead to aggregation and poor assembly of intermediate filaments such as keratin 10 in keratinocytes [6] and vimentin in fibroblasts [10]. As a result, instability of the cytoskeleton, cell shape and defects in migration, cellular division, and contraction occur. Various other intracellular proteins including enzymes may be targeted by glycation. Moreover, glycation of enzymes of the ubiquitin proteasome system and of the lysosomal proteolytic system has been shown to inhibit their function [40]. Antioxidant and other protective enzymes such as Cu-Zn-superoxide dismutase (Cu-Zn-SOD) can be inactivated [41]. Other intracellular components, such as DNA and lipids can be glycated with detrimental effects on their function [42].

Growth Factors and Growth Factor Receptors

AGE-adducts of growth and their receptors can alter their binding affinities and impair their signal transduction properties. Glycated basic fibroblast growth factor (bFGF) displays impaired mitogenic activity in endothelial cells [43]. Dicarbonyls such as glyoxal and methylglyoxal also alter signaling of epidermal growth factor receptor (EGFR), a receptor controlling various cellular functions such as proliferation, differentiation, motility and survival, by formation of EGFR crosslinks, blocking of phosphorylation and impaired activation of ERKs and phospholipase C (PLC) [44].

Cutaneous Cell Types Responding to AGEs

RAGE is expressed by various cell types of human skin including keratinocytes, dermal fibroblasts, and endothelial cells as shown by in vitro cell culture studies. In situ RAGE expression is abundant in sites of solar elastosis and its expression is enhanced by AGEs and proinflammatory cytokines such as TNF-α [27].

AGEs have been shown to affect various cellular functions in vitro (Table 2). In keratinocytes, AGEs impair cell differentiation [46], induce senescence [46], decrease cell viability [46] and
migration [45], and induce MMP expression [45], partly via enhanced NF-κB signaling [48]. Furthermore, they decrease expression of β-defensin-2 and -3 leading to increased susceptibility to infections [53]. Recently, S100 A8/A9-RAGE interaction has been implicated in the pathogenesis of squamous cell carcinomas in human skin [54].

AGEs furthermore decrease proliferation and induce apoptosis of human dermal fibroblasts, an effect which is mediated via RAGE-signaling and leads to transcription of various proapoptotic genes and activation of NF-κB and caspases-3, -8, and -9 [49]. Expression of beta-galactosidase, a marker of senescence, is induced [51]. Collagen and ECM protein synthesis have been also found to be decreased, while the expression of MMPs is induced by AGEs [50].

In endothelial cells, AGEs increase expression of proinflammatory cytokines and chemokines like TNF-α, IL-6, and MCP-1 [52]. Moreover, they increase expression of adhesion molecules like VCAM-1 and increase vascular permeability [52]. Furthermore, AGE-modification of bFGF decreases mitogenic activity of these cells [43].

In the context of photoaging, UVA irradiation in the presence of AGEs increases ROS production and decreases viability of both epidermal keratinocytes [47] and dermal fibroblasts [55].

### AGEs and ROS

ROS – generated during normal cell metabolism throughout lifetime and also increasingly propagated via UV exposure (photoaging) and other...
noxious stimuli – are central mediators of intrinsic and extrinsic skin aging. Importantly, in vitro exposure of AGEs to UVA leads to ROS formation such as superoxide anion, hydrogen peroxide, and hydroxyl radicals [55]. AGEs can lead to ROS formation within cells by various pathways. Firstly, they can stimulate NADPH oxidase in various cell types including human dermal fibroblasts, endothelial cells, and in immune cells at least partly via interaction with RAGE [24]. Activation of the catalytic subunits NOX1 [56], NOX2 [57], NOX 4 [58] as well as of the regulatory subunit p47phox [59] in a cell-specific manner has been reported. Secondly, AGEs can suppress antioxidative defense systems including Cu-Zn-SOD which is inactivated by crosslinking and site-specific fragmentation of the Cu-ZnSOD molecule [41]. During these crosslinking reactions, AGEs can directly act as electron donors leading to formation of superoxide anion [60]. Finally, AGEs with chromophores can act as endogenous photosensitizers leading to increased ROS formation after UVA irradiation of human skin.

**Anti-AGEs Strategies as a Perspective Against Skin Aging**

Since AGEs play an important pathogenetic role in aging, substances able to inhibit formation of AGEs, to break already formed AGEs, or to antagonize their signaling could have a beneficial effect on skin aging as well as on age-related skin diseases. Until now various substances have been proposed and some of them are already being tested in clinical trials [61].

Aminoguanidine, one of the first identified substances, is a nucleophilic hydrazine which traps and inactivates early glycation products like dicarbonyl compounds. Although it showed promising results in various in vivo animal models, further drug development was stopped after a phase III clinical trial showed significant toxicity [62]. Notably, the anti-AGE properties of aminoguanidine have been demonstrated in in vitro and tissue-engineered skin models but in vivo data of this chemical on human skin are lacking [31, 63].
Pyridoxamine is a naturally occurring vitamin B6 isoform which traps reactive carbonyl intermediates and scavenges ROS. Oral intake of pyridoxamine inhibited CML formation in skin collagen of diabetic rats [64]. However, its potential against skin aging remains to be shown.

“AGE-breakers” are chemical substances which recognize and break the Maillard reaction crosslink via a thiazolium structure like dimethyl-3-phenacyl-thiazolium chloride (ALT-711), N-phenacylthiazolium, and N-phenacyl-4,5-dimethylthiazolium [65]. Of note, topical ALT-711 application improved skin hydration in a rat aging model [65].

Interference with intrinsic AGE-detoxification enzymes like Glo I and II, fructosamine kinases, and FAOXs could be a further strategy against AGEs as these enzymes recognize specific substrates and their inhibition may be associated with fewer side effects compared with the above chemicals. Interestingly, it has been shown that Glo I is transcriptionally controlled by Nrf2 and that pharmacological Nrf2 activators increase Glo I mRNA and protein levels as well as its activity [66]. Therefore, pharmacological activation of the Nrf2 pathway, e.g., by electrophils or other compounds, may lead to increased expression of Glo I and could have beneficial effects on skin aging by reducing the amounts of deposited AGEs.

Since oxidation reactions are involved in many steps of AGEs formation, substances with antioxidative or metal chelating properties may act as AGE-inhibitors. A lot of interest has been therefore directed to nutrients and vitamins, so-called nutraceuticals, as natural weapons against AGEs [61]. The list of such substances is

![Fig. 2 Effects of AGEs in skin](image-url)
long: ascorbic acid, α-tocopherol, niacinamide, pyridoxal, rivoflavin, zinc, α-lipoic acid, green tea, vitamins C and E, N-acetylcyesteine, taurine, spices and herbs like ginger, cinnamon, cloves, and marjoram, and naturally occurring flavonoids such as luteolin, quercetin, and rutin have all shown antiglycating properties mostly in vitro but also in animal models [67, 68]. A blueberry extract, an AGE-inhibitor and C-xyloside, a glycosaminoglycan (GAG) synthesis stimulator, was tested in female diabetic subjects for 12 weeks and showed significant improvement on firmness, wrinkles, and hydration of skin although they failed to show a significant decrease in the cutaneous content of AGEs [69]. Recently, oral intake of a mangosteen extract showed some reduction in AGE-related skin autofluorescence and improved skin hydration as well as elasticity in healthy volunteers [70].

Restriction of intake of dietary AGEs may be perhaps the simplest strategy to prevent accumulation of AGEs in the body and to limit their deleterious effect. Dietary glycotoxins significantly increase concentrations of systemic inflammatory mediators like TNF-α, IL-6, and CRP [4]. A diet with a low content in AGEs was shown to reduce circulating AGEs and inflammatory biomarkers in patients with diabetes and renal failure [71]. In mice, low dietary AGEs had beneficial effects on wound healing [72]. There are no studies investigating the effects of AGE-poor diets on skin aging in humans. However, it has been shown that skin collagen glycation positively correlates with blood glucose levels in diabetes and that intensive treatment can reduce the levels of skin glycation [73], implicating that a diet low in AGEs may have a beneficial effect on skin glycation.

Antagonism of AGEs at the receptor level would be another potential strategy against AGEs. Interesting effects in various systems have been shown in vitro and in vivo by neutralizing RAGE or with small-molecule RAGE inhibitors [74]. Moreover, protective effects of sRAGE, the natural soluble decoy receptor of AGE, have been reported in diabetes and inflammatory models [23, 24]. Accordingly, sRAGE attenuates impaired wound healing in diabetic mice [75]. Further studies will be needed to investigate if these antibodies and small molecules have also promising effects in preclinical models of skin aging.

Finally, topical application of molecular chaperones such as carnosine and carnitine has shown an improvement of skin appearance which appeared to be at least in part mediated by lowering cutaneous accumulation of AGEs [76].

**Conclusion**

There is clear evidence that AGEs affect many targets in the skin via receptor- and nonreceptor-mediated pathways (Figs. 1 and 2). These cellular and noncellular targets are important players in the context of skin aging. In light of the intimate connection between ROS and AGEs glycation therefore appears to be an important contributor to cutaneous aging. Until now, there are several studies that assessed the value of anti-AGE strategies in patients with diabetes. As clinical trials on the effects of these anti-AGE strategies on skin aging are still missing, this could be a promising field in the future to further promote healthier skin aging.

**References**


