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COMPREHENSIVE INVITED REVIEW

Hyperglycemia and Glycation in Diabetic Complications

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Abstract

Diabetes mellitus is a multifactorial disease, classically influenced by genetic determinants of individual susceptibility and by environmental accelerating factors, such as lifestyle. It is considered a major health concern, as its incidence is increasing at an alarming rate, and the high invalidating effects of its long-term complications affect macro- and microvasculature, heart, kidney, eye, and nerves. Increasing evidence indicates that hyperglycemia is the initiating cause of the tissue damage occurring in diabetes, either through repeated acute changes in cellular glucose metabolism, or through the long-term accumulation of glycated biomolecules and advanced glycation end products (AGEs). AGEs represent a heterogeneous group of chemical products resulting from a nonenzymatic reaction between reducing sugars and proteins, lipids, nucleic acids, or a combination of these. The glycation process (glucose fixation) affects circulating proteins (serum albumin, lipoprotein, insulin, hemoglobin), whereas the formation of AGEs implicates reactive intermediates such as methylglyoxal. AGEs form cross-links on long-lived extracellular matrix proteins or react with their specific receptor RAGE, resulting in oxidative stress and proinflammatory signaling implicated in endothelium dysfunction, arterial stiffening, and microvascular complications. This review summarizes the mechanism of glycation and of AGEs formation and the role of hyperglycemia, AGEs, and oxidative stress in the pathophysiology of diabetic complications. *Antioxid. Redox Signal.* 11, 3071–3109.

I. Introduction

Diabetes mellitus is a major cause of morbidity and mortality and is considered a worldwide economic problem, because it affects nearly 8% of the population in the world. Diabetes is characterized by hyperglycemia resulting from quantitative deficiency in insulin secretion or from insulin resistance. Diabetic metabolic changes result in macrovascular complications leading to accelerated atherosclerosis and coronary heart or peripheral arterial disease or both, and in microvascular complications leading to retinopathy, nephropathy, and neuropathy (143).

Several clinical prospective trials designed for investigating whether diabetes complications are related to glycemia regulation (the Diabetes Control and Complications, DCCT; the United Kingdom Prospective Diabetes Study, UKPDS) clearly established that hyperglycemia causes vascular and tissue damage in patients with diabetes (1, 2), which can be worsened by genetic factors of individual susceptibility and by associated pathologic factors, such as hypertension or hyperhomocysteinemia. It is generally admitted that repeated acute changes in blood glucose and cellular glucidic metabolism, as well as cumulative long-term alterations of cellular and extracellular constituents, represent the mechanisms that mediate the damaging effects of hyperglycemia. A strict glycemic control may reduce in part the incidence of microvascular complications but is less effective in preventing the progression of macrovascular diseases that are largely associated with hypertension (105). For this reason, Schmidt and colleagues (355) proposed the hypothesis of the "lasting glycemic memory," resulting from the accumulation of advanced glycation end products (AGEs) and from their interactions with their specific receptor RAGE, as a trigger for sustained and progressive vascular complications. The damaging effects of hyperglycemia affect mainly certain cell types that (a) are unable to maintain their intracellular glucose concentration in hyperglycemic conditions, and particularly cell types involved in diabetic complications (endothelial cells in the vascular system, mesangial cells in the kidney, neurons and neuroglia in the nervous system, and pancreatic β cells); and (b) generate high reactive oxygen species (ROS) levels and induce a huge oxidative stress implicated in glycation, AGEs formation, and AGE/RAGE interactions.

This review focuses on the mechanisms leading to acute glycemic changes and chronic hyperglycemia complications, with special emphasis on the implication of oxidative stress, metabolic and chemical formation of AGEs, AGE/RAGE signaling, and their general consequences in diabetes complications.

II. Oxidative Stress and Metabolic Dysfunction in Acute Hyperglycemia and Diabetes

A. Oxidative stress: Generalities

Reactive oxygen species (ROS) are normally produced throughout oxygen metabolism (Table 1) and are considered major players in basal and pathologic cell signaling (a process named redox signaling) (305). Several sources of ROS are identified, particularly the mitochondrial respiratory chain and oxidative enzymes such as xanthine oxidase, lipoxygenases, NADPH oxidases (NOXs), redox-active flavoproteins, cytochromes P450, and other oxidases (305). In addition to their production rate, ROS levels (and their biologic effects) are regulated by the antioxidant ability of biologic systems to neutralize, detoxify, and repair the damage caused to cells by ROS. The cellular antioxidant systems include low-molecular-weight antioxidants (*e.g.*, ascorbic acid, glutathione, tocopherols, uric acid), and "antioxidant" enzymes, such as superoxide dismutase (SOD), glutathione peroxidases, and catalase, which contribute to maintaining a normal intracellular redox homeostasis. Some enzymes may act indirectly as antioxidants: for instance, heme-oxygenase reduces the vascular oxidative stress in experimental diabetes, by increasing the expression of extracellular SOD (EC-SOD) and catalase (330). It may be noted that antioxidant efficiency requires their availability in the subcellular compartment where ROS are generated (103).

Oxidative stress results from the imbalance between ROS production and cellular antioxidant defenses. In diabetes and its complications, oxidative stress results from an overproduction of ROS, generated by glucose autoxidation, mitochondria dysfunction, polyol pathway, and protein glycation

Table 1. Some Radical and Nonradical Reactive Oxygen and Nitrogen Species in Living Organisms

Radicals		
Superoxide	$O_2^{\circ -}$	Formed by NADPH oxidase and electron-transport chain. Dismutated to H_2O_2 by SOD. Widely implicated in biologic reactions and cell signaling
Hydroxyl	OH°	Formed by Fenton reaction and ONOO decomposition. Very reactive and toxic
Peroxyl	ROO^{-}	Formed by radical addition to double bonds in presence of O_2. Implication in lipid peroxidation reactions
Thyl	RS°	Formed by autooxidation of thiols in the presence of metals
Nitric oxide	NO°	Biosynthesized from arginine and oxygen by NO synthases (NOS). Key biologic messenger in a variety of biologic processes
Nitrogen dioxide	NO_2°	Formed by oxidation of NO° by O_2. Air pollutant
Nonradicals		
Peroxynitrite	$ONOO^{-}$	Formed in a rapid reaction between $O_2^{\circ -}$ and NO°. Lipid soluble
Hypochlorite	ClO^{-}	Formed from H_2O_2 and chloride ions by myeloperoxidase. Lipid soluble. Highly reactive
Hydrogen peroxide	H_2O_2	Formed by SOD-mediated $O_2^{\circ -}$ dismutation or by reduction of O_2. Lipid soluble. Diffusion across membranes. Major cell-signaling agent
Singlet oxygen	$^1O_2$	Less-stable diamagnetic form of molecular O_2. Lipid peroxidation
Lipid peroxide	LOO^{-}	Formed by oxidation of PUFA in the presence of O_2. Lipid peroxidation

(reviewed by Ceriello) (46), and from decreased antioxidant defenses and plasma antioxidants.

B. Acute hyperglycemia, glycemia variability, and oxidative stress

In normal subjects, glycemia is maintained in a relatively narrow range from 2.5 to 5.5 m*M* in the fasting state. Acute and postprandial hyperglycemia could trigger oxidative stress in healthy subjects (198), and recent data suggest that not only sustained hyperglycemia, but also glycemic variability, are determinant factors of diabetic complications *via* oxidative stress induction (213).

1. Acute hyperglycemia and oxidative stress *in vivo*.

Acute hyperglycemia in diabetes is characterized by an increase of biomarkers of oxidative stress and cytokine secretion (92), which are concomitant with a decreased activity of antioxidant systems induced by glucose intake.

After intake of glucose and fructose, the level in F_2-isoprostanes (a biomarker of oxidative stress) increases concomitant with the decrease in plasma total antioxidant defenses, suggesting a participation of the Maillard reaction (fructose being more reactive than glucose) (95, 284). The levels of isoprostanes are well correlated with glucose fluctuations detected by continuous glycemic monitoring, even in the absence of the increase of mean glycemia or HbA1c, in healthy individuals and type 2 diabetes patients (213). Similarly, *in vitro* experiments show that oxidative-stress biomarkers are higher in cells exposed to changes between high and low glucose—without reaching hypoglycemia levels—than in cells exposed to continuously high levels of glucose (269).

Among the other oxidative-stress biomarkers, circulating thioredoxin is decreased after glucose loading in humans (211), whereas it is often stated that thioredoxin-level increases are associated with oxidative stress (39). However, oxidative modification of thioredoxin could be responsible for its decrease after glucose intake.

Concerning the intracellular redox status, an increased plasma ascorbate concentration has been reported in type 2 diabetes patients after glucose intake (198), without any changes in NO, superoxide ($O_2^{\circ -}$) or hydrogen peroxide (H_2O_2) status in leucocytes.

Experiments in humans, by using euglycemic and hyperglycemic clamps, suggest that transient increases in glycemia result in $O_2^{\circ -}$ generation in endothelial cells, not enough elevated for generating peroxynitrite ($ONOO^{-}$), but high enough to be sensed by cellular response systems, and leading to increased NO release and vasodilation (198). This suggests that transient hyperglycemia could contribute to vasodilation, whereas more-sustained hyperglycemic states would generate more superoxide, interaction with NO and formation of $ONOO^{-}$, and nitrosylation of tyrosine residues of proteins (198).

Conflicting results have been reported about the plasma antioxidant status, concerning ascorbate, allantoin (derived from uric acid oxidation), and malondialdehyde changes subsequent to glucose intake (48). An oral glucose challenge and usual meal consumption could induce a decrease in antioxidant defenses (49), but a recent review stated that some of the limitations of the work relates to acute increases in glycemia and oxidative stress, (in particular, the choice of markers and of models for oxidative stress assessment) (61). The relation between acute hyperglycemia and oxidative stress is supported by therapeutic approaches with antidiabetes drugs, such as sulfonylureas, meglitinide, biguanides, and thiazolidinediones, which clearly decrease oxidative stress (116). However, no direct controls were done on the antioxidant action *per se* of the compounds, precluding a causal analysis (61, 293).

2. High glucose induces ROS generation in cultured cells.

Although culture conditions are far from physiologic conditions (high glucose concentration in a majority of culture medium, high O_2 pressure under standard culture conditions), cell cultures have been used for investigating the effects of high glucose on cell physiology. Cultured endothelial and mesangial cells grown in normal (5 m*M*) and placed in high glucose (25–30 m*M*) medium exhibit an increase of intracellular ROS (124, 234) and repeated fluctuations of glucose concentration (between 5 and 25 m*M*) on cultured cells also induce a higher ROS increase than that induced by constant high glucose level (243). However, the increase in ROS production depends on glucose uptake and metabolism that can be highly different according to the cell type and the cell lines (61).

The high glucose–induced ROS generation may originate from various cellular sources, but mitochondria are apparently the initial generator of ROS production, as suggested by the inhibitory effect of Mn-SOD and UCP-1 overexpression (234, 352). The increase of cellular ROS induced by high glucose can mediate several cellular responses of hyperglycemia (178).

However, the pathophysiologic relevance of these investigations is limited because of the nonphysiologic conditions of cell culture, because number of culture media contain high glucose concentration (25 m*M*), thereby adapting cells to high glucose under basal conditions, and because of high O_2 concentration (21%), which is largely different from the *in vivo* conditions.

Many factors, potentially related to vascular diseases in diabetic complications, are influenced by acute glycemia [reviewed by Ceriello *et al*. (47)]: LDL oxidation, decreased production of endothelial NO, increased formation of $ONOO^-$, increased coagulability, and increased circulating levels of ICAM-I, IL-6, IL-18, and TNF-α. These responses are prevented by antioxidants, which supports a pathogenic role for oxidative stress subsequent to glycemia changes in diabetes complications.

C. *Chronic hyperglycemia and oxidative stress*

1. Hyperglycemia and mitochondrial free radical production. As described earlier, increased generation of ROS is a common feature of cells damaged by hyperglycemia (86, 234). In living cells, the major sites of physiologic ROS generation are the complexes I and III of the mitochondrial electron-transport chain, which contain several redox centers (flavins, iron–sulfur clusters, and ubisemiquinone) capable of transferring one electron to O_2 to form $O_2^{\cdot -}$ (27, 145, 245) (Fig. 1). ROS generation by complexes I and III is dependent on the degree of electronic reduction of these generators. More precisely, when the NADH/NAD+ ratio is increased (high energy supply), the degree of reduction of complex I increases, and ROS production also increases (245). As mitochondrial ROS production is very sensitive to the proton motive force, mild uncoupling of mitochondria (for instance, by expression of uncoupling proteins (UCPs)) reduces ROS generation (27, 232).

In diabetic cells, the amount of glucose being oxidized in the Krebs tricarboxylic acid cycle is increased, which pushes more electron donors (NADH and $FADH_2$) into the electron-transport chain, leading to an increase in ROS generation (34, 85, 234, 245). Conversely, in insulin resistance, the increased free fatty acid (FFA) flux from adipocytes into arterial endothelial cells results in increased FFA oxidation by mitochondria (2, 35). Interestingly, fatty acids β-oxidation and oxidation of acetyl-CoA by the TCA cycle generate the same electron donors (NADH and $FADH_2$) as does glucose oxidation. This suggests that excessive FFA oxidation reinforces the

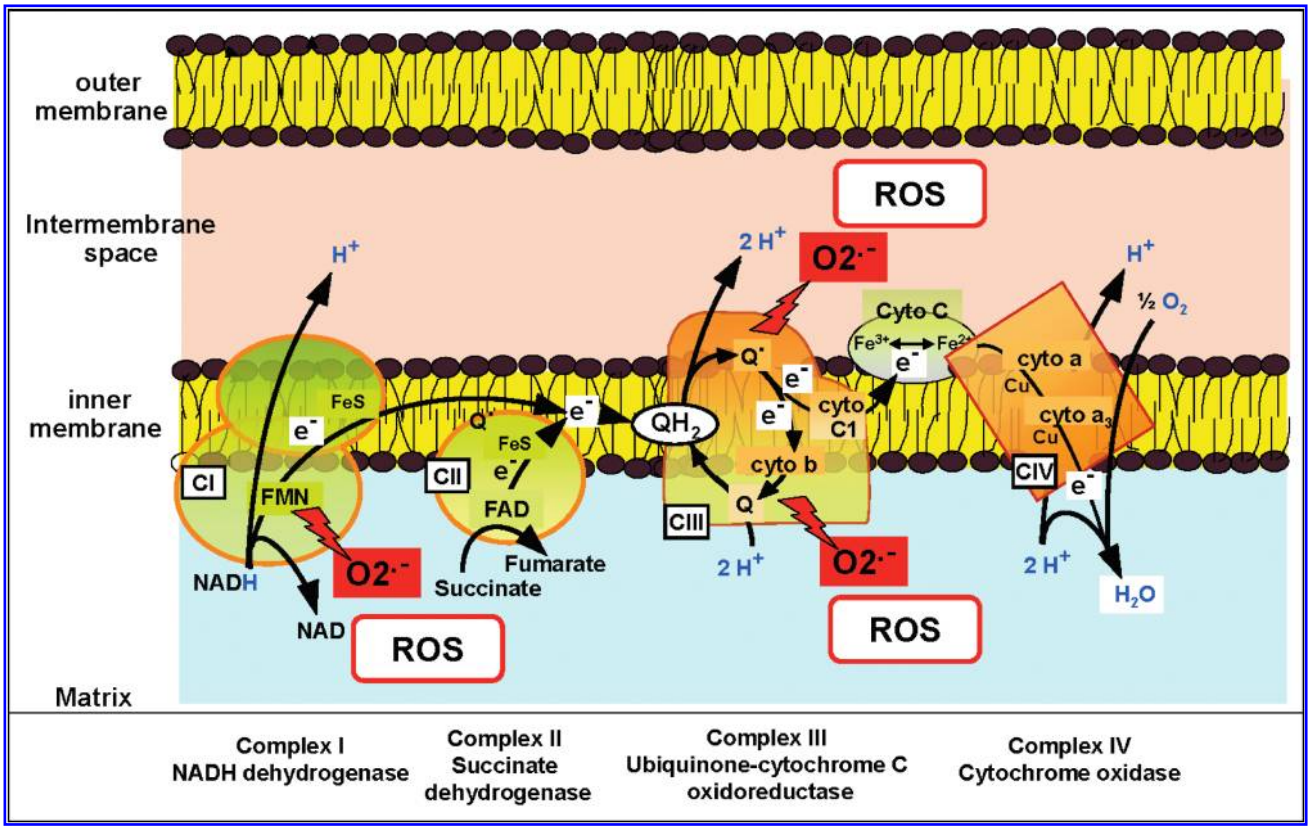

FIG. 1. ROS generation by the mitochondrial respiratory chain. Schematic representation of the mitochondrial complex I and complex III as main free-radical generators. Several physiologic mechanisms influencing the rate of mitochondrial ROS generation include (a) the degree of electronic reduction of these generators; and (b) the uncoupling proteins. Oxygen radicals attack lipids, carbohydrates, proteins, and DNA (modified from ref. 145). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

mitochondrial overproduction of ROS by high glucose, thereby worsening the oxidative stress.

2. ROS in the pathogenic mechanisms of diabetes complications. A recent unifying theory from Brownlee (35) suggests that ROS may constitute a common trigger of the various biologic effects occurring in diabetes.

From a chronologic point of view, the polyol pathway is the first metabolic mechanism described in diabetes (113). A second mechanism is represented by the nonenzymatic formation of advanced glycation end products (AGEs) (18, 38, 216). In the 1990s, two other pathways were described, the hyperglycemia-induced activation of protein kinase C (PKC) (170) and the hexosamine pathway and consequent overmodification of proteins by *N*-acetylglucosamine (167).

The polyol pathway focuses on the enzyme aldose reductase (75). The aldose reductase catalyzes the reduction of toxic aldehydes to inactive alcohols, but is also capable of reducing glucose to sorbitol. This pathway, which operates mainly in case of high glucose concentration, consumes NADPH, a cofactor of aldose reductase (91, 177). By reducing the level of NADPH, the polyol pathway induces a decrease of the cellular capacity to regenerate reduced glutathione, an essential regulator of the cellular redox equilibrium, and finally increases the risk of intracellular oxidative stress. Moreover, sorbitol is metabolized into fructose, fructose-1-phosphate, which can be split into glyceraldehyde, and dihydroxyacetone phosphate. Glyceraldehyde can be metabolized into glyceraldehyde-3-phosphate, thus returning to the glycolysis, or it can react with amino groups, thereby participating in AGEs formation. The metabolism of the other product, dihydroxyacetone phosphate, results in phosphatidic acid and diacylglycerols.

The activation of the PKC pathway by high intracellular glucose is mediated by diacylglycerols, which act as coactivators for several isoforms, such as PKC-β, -δ, and -α (170, 346). Diacylglycerols can also result from diacylglycerophospholipid hydrolysis by phospholipase C. Activation of PKC triggered by high intracellular glucose participates in altering the function of several regulatory pathways and the expression of a variety of genes, including downregulation of endothelial NO synthase (eNOS), upregulation of endothelin-1, transforming growth factor-β (TGF-β), plasminogen activator inhibitor-1 (PAI-1), and vascular endothelial growth factor (VEGF) activation of NF-κB and NADPH oxidase. These events contribute to increase oxidative stress and inflammatory responses and lead to cellular dysfunction and pathophysiologic responses, such as increased collagen synthesis and fibrosis, abnormal capillary permeability, blood-flow abnormalities, abnormal angiogenesis, vascular remodeling, and stenosis (35).

The hexosamine hypothesis results from the observation that a high glucosamine level leads to metabolic changes similar to those occurring in type 2 diabetes (204). In the early steps of glycolysis, glucose is converted into glucose-6 phosphate, and then into fructose-6-phosphate, which is also the initial step of biosynthesis of *N*-acetylglucosamine. Indeed, fructose-6-phosphate is converted into glucosamine-6 phosphate and finally to UDP-*N*-acetyl glucosamine, UDP-GlcNAc, which is then used for the biosynthesis of glycoproteins, proteoglycans, and glycolipids. O-GlcNAc glycosylation on serine or threonine is a ubiquitous post-translational protein modification that takes part in sensing the nutrient status of the cell and controls the localization, the activity, and the stability of cytosolic and nuclear proteins. High glucose leads to increased O-GlcNAc glycosylation of cellular proteins, including transcription factors, and induces gene dysregulation (35). For instance, high glucose enhances the expression of PAI-1 and TGF-β through hexosamine-dependent modification of sp1 and mSin3a, transcription factors (358). Finally, overexpression of GFAT (glutamine: fructose-6 phosphate amidotransferase, which converts the fructose-6 phosphate to glucosamine-6 phosphate) in transgenic animals, leads to insulin resistance, excessive synthesis of fatty acid by the liver, and high secretion of insulin by beta cells, leading to hyperinsulinemia (35, 204). This suggests that overproduction of glucosamine-6 phosphate subsequent to high intracellular glucose may induce the metabolic features of type 2 diabetes (35).

Brownlee (35) proposed an interesting unifying scenario, in which the oxidative stress induced by hyperglycemia and high intracellular glucose orchestrates the metabolic dysregulation. In these events, poly(ADP-ribose) polymerase (PARP) plays a crucial role. Oxidative stress induces DNA damages, thereby activating enzymes of the PARP family, which catalyzes a posttraductional modification of proteins by adding adenosine diphosphate (ADP)-ribose (by cleavage of NAD). ADP-ribosylation of cellular proteins by PARPs participates in the regulation of a variety of biologic processes (for instance, repair of single-strand DNA nicks, spindle assembly during mitosis, vesicular trafficking of GLUT4 vesicles, inflammation, and enzymes involved in intermediate metabolism). During diabetes, high glucose induces overproduction of ROS by the mitochondrial electron-transport chain that can be prevented by overexpression of UCP-1 or of MnSOD (84). DNA-strand breakage resulting from oxidative stress activates PARP, which catalyzes the ADP-ribosylation of glyceraldehyde-3 phosphate dehydrogenase (GAPDH), and thereby downregulates the activity of this key enzyme of glucidic metabolism (35). Inhibition of GAPDH by ADP-ribosylation probably represents an inhibitory mechanism of glycolysis and of the subsequent metabolite flux to mitochondria, allowing a decrease of ROS production (Fig. 2). GAPDH inhibition induces an increase of glyceraldehyde-3 phosphate (GAP), in equilibrium with dihydroxyacetone phosphate (DHAP). GAP can be metabolized into methylglyoxal (MGO), an AGEs precursor, and DHAP is a substrate for the biosynthesis of lipids, including diacylglycerols, that activate PKC (34, 35) (Figs. 2 and 3).

III. Glycation and AGEs: Formation, Excretion, and Biologic Effects

Glycation (sometimes termed nonenzymatic glycosylation) was discovered by LC Maillard (195). To avoid any confusion, the term glycation rather than nonenzymatic glycosylation should be preferred. It is the chemical (nonenzymatic) reaction of reducing sugars with amino groups. Glycation generally impairs the function of biologic molecules, in contrast to enzymatic glycosylation of proteins, which occurs in endoplasmic reticulum and Golgi and is required for obtaining functional glycoproteins.

The initial glycation reaction is followed by a cascade of chemical reactions resulting in the formation of intermediate products (Schiff base, Amadori and Maillard products) and

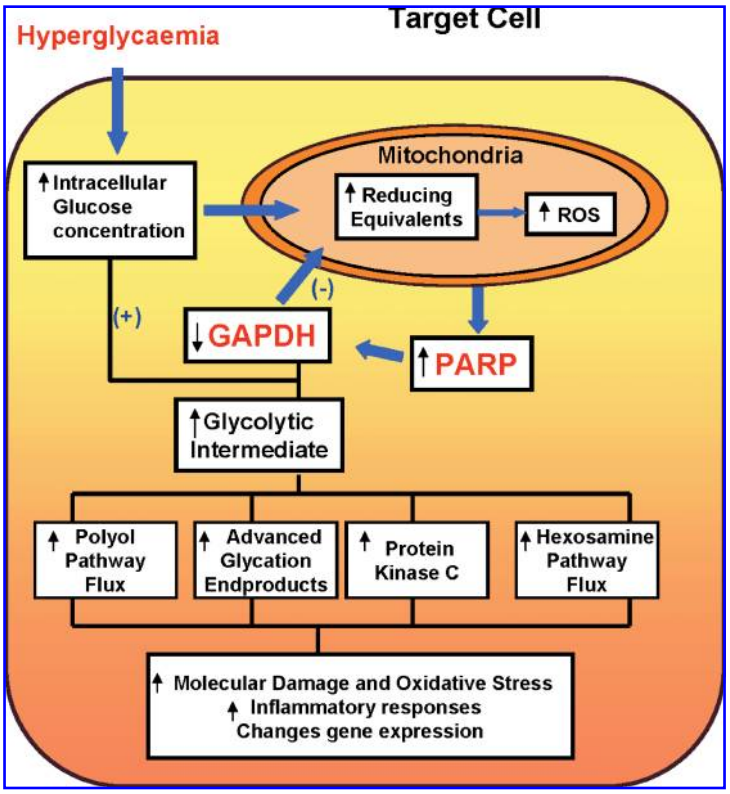

FIG. 2. Intracellular high-glucose metabolism and oxidative stress. When intracellular glucose concentration increases in target cells of diabetes complications, it causes increased mitochondrial production of ROS and activates PARP. PARP then modifies GAPDH, thereby reducing its activity. Finally, decreased GAPDH activity increases glycolytic intermediates that, in turn, activate the polyol pathway, increases intracellular AGEs formation, activates PKC and subsequently NF-κB, and activates hexosamine pathway flux. All these metabolic pathways elicit alterations in gene expression, inflammatory responses, and structural and functional changes in cellular constituents that represent the molecular basis of the diabetic process (modified from ref. 35). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

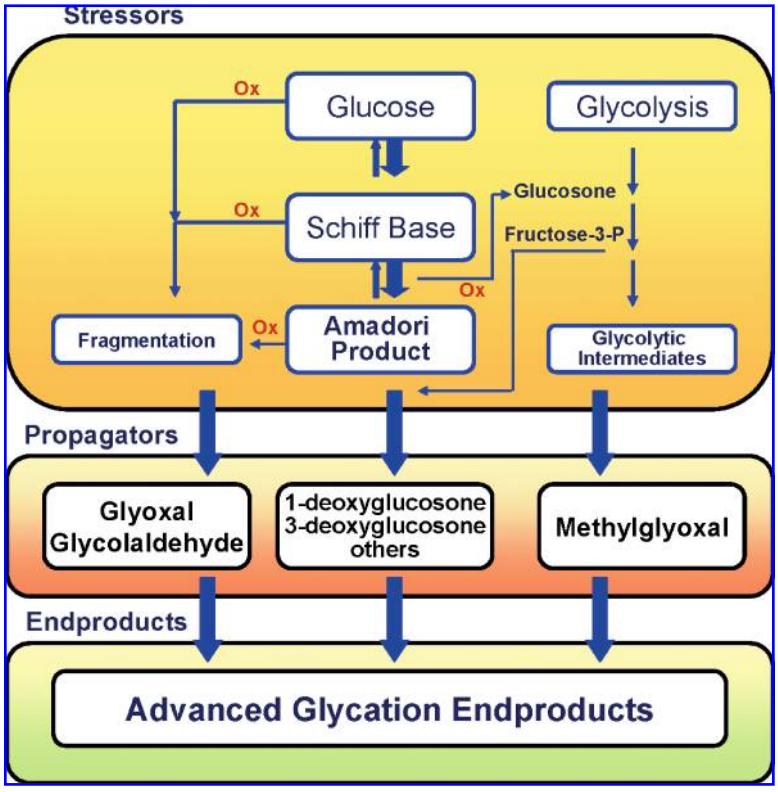

FIG. 3. Biochemical pathways for AGEs formation. The glycation process starts by a nucleophilic addition reaction between a free amino group from a protein (or other molecule) and a carbonyl group from glucose (or a reducing sugar) to form a Schiff base, which can rearrange into Amadori products that undergo a series of reactions, including the formation of glucosones, oxidant-induced fragmentation leading to the formation of short-chain reactive compounds, such as GO, glycolaldehyde, and MGO, which can also be generated by glycolytic pathways, and during lipid peroxidation. All these reactive compounds may react with amino groups of biologic molecules, and generate AGEs (modified from ref. 214). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

finally to a variety of derivatives named advanced glycation end products (AGEs).

A. Glycation and AGEs formation

1. Chemical mechanisms of glycation and AGEs formation. Reducing sugars (glucose, galactose, fructose, ribose), intermediates of glucidic metabolism (glucose-6-phosphate, fructose-6-phosphate, ribose-5-phosphate, and desoxyribose-5-phosphate, glyceraldehyde), metabolites of the polyol pathway (fructose or fructose 3-phosphate), and carbonyl compounds are able to react with amino groups of biologic molecules to form AGEs (87). It may be noted that glucose is less efficient than the other reducing monosaccharides (307).

Glycation is a spontaneous concentration-dependent reaction starting with a nucleophilic addition between a free amino group (from protein or other molecule) and a carbonyl group from glucose (or a reducing sugar) to form a reversible Schiff base. Then the Schiff base can rearrange into Amadori products (Fig. 3). Alternatively, Amadori adducts may be fragmented by glycoxidation, in the presence of transition metal ions, leading to the formation of short-chain reactive compounds, such as glyoxal (GO) and methylglyoxal (MGO).

Table 2. Levels of Fructoselysine (Amadori Compound) in Mammalian Tissues

Protein-tissue/specimen	*Levels*	*Method*	*Ref.*
Albumin			
Human	250–500 pmol/mg	RIA	230
Diabetic	400–1,500 pmol/mg		
Albumin			
Human	3–7 nmol/mg	ELISA	229
Diabetic	7–24 nmol/mg		
Plasma protein			108
Human	1.1 ± 1.6 nmol/mg[a]	Furosine HPLC	
Peritoneal fluid			
Human	1.2 ± 2.1 nmol/mg[a]		
LDL			109
Human	0.76–0.80 mmol/mol lys	Furosine SIM-GC/MS	
Lens crystallins			
Human	0.5–2.0 mmol/mol lys (no change with age)	Furosine SIM-GC/MS	87
Dog			
Normal	100 ± 20 pmol/mg[a]	Furosine HPLC	223
Diabetic (moderate)	510 ± 620 pmol/mg[a]		
Diabetic (severe)	$2{,}250 \pm 680$ pmol/mg[a]		
Skin collagen			
Human			
Normal	2.5–5.0 mmol/mol lys	Furosine SIMS-GC/MS	89
Diabetic (type 1)	5–25 mmol/mol lys (very small age change)		
Human			
Normal	400 ± 100 pmol/mg[a]	Furosine HPLC	215
Diabetic (type 1)	920 ± 250 pmol/mg[a]		192
Diabetic (intense therapy)	600 ± 75 pmol/mg[a]		
Rat (Brown Norway)			
Normal	1.7–2.1 mmol/mol lys	Furosine SIMS-GC/MS	45
Dietary restriction	1.2–1.4 mmol/mol lys		
Rat (Fischer 344)			
Normal	290–450 pmol/mg[a]	Furosine HPLC	296
Dietary restriction	290–350 pmol/mg[a] (from 5 to 25 mo)		
Mouse (C57BL)			
Normal	100–500 pmol/mg[a]	Furosine HPLC	296
Dietary restriction	100–350 pmol/mg[a] (from 2 to 25 mo)		
Hemoglobin			
Human	$50.7 \pm 6.2\ \mu M$	HMF-TBA	266
Kidney (rat)			
Normal	0.25 ± 0.08 mmol/mol lys	Furosine SIM-GC/MS	263
Diabetic	2.90 ± 0.18 mmol/mol lys		
Liver (rat)			
Normal	0.52 ± 0.07 mmol/mol lys		
Diabetic	2.59 ± 0.09 mmol/mol lys		
Liver aminophospholipids			
Rat			
Normal	3.43 ± 0.5 pmol	HPLC	246
Diabetic	HMF/μmol PL 9.01 ± 1.90 pmol HMF/μmol PL		

[a]Furosine values were not corrected for a 70% loss during acid hydrolysis.

GO and MGO also are generated by accessory glycolytic pathways and during lipid peroxidation. All these reactive compounds react with amino groups of biologic molecules, thus generating a wide variety of adducts and cross-links called AGEs (6, 259, 326). These studies, initially centered in proteins, have been extended to other biologic molecules containing amino groups, such as DNA (17) and aminophospholipids (249).

2. Exogenous glycation and AGEs formation. The Maillard reaction (glycation and AGEs formation) is a complex chemical (nonenzymatic) reaction between sugars and amino groups-containing molecules (proteins, amino acids, aminolipids, nucleic acids), which is accelerated by food heating, at alkaline pH, and low water concentration. This reaction results in a multitude of products participating in the formation of brown pigments and volatile aromatic compounds of cooked foods.

AGEs-modified proteins are partially resistant to proteolysis and their nutritive value is reduced because dietary AGEs are only poorly digested and absorbed. AGE derivatives are present in the blood (7, 93, 169) and are excreted in the urine by healthy subjects (102). However, in patients affected with (diabetic) renal failure, this excretion is deficient, and an AGEs-rich diet can aggravate AGEs-mediated cellular dysfunctions and tissue damage. Exogenous AGEs have long been regarded as unimportant contributors to diseases, but recent studies have shown that they may add their own pathogenic effects to endogenous AGEs.

3. Endogenous glycation and AGEs formation. Proteins are classic targets for the Maillard reaction *via* the formation of Schiff bases and Amadori adducts (326), 3-deoxyglucosones (1DG, 3DG) (Table 2), GO, and MGO. These compounds react with cellular constituents to form AGEs directly (Figs. 3 and 4). The dicarbonyl compounds have a relatively long half-life (minutes to hours) and easily cross the plasma membrane, thereby acting far from their production site and modifying target molecules both inside and outside. The accumulation of dicarbonyl compounds, issued from glycoxidation or lipoxidation or both is called "carbonyl stress." Their concentration is significantly increased in diabetes patients (Tables 3 and 4).

Chemically, reactive carbonyl compounds can react with lysine, arginine, histidine, or cysteine residues of proteins through complex chemical mechanisms, sometimes involving an oxidation step (glycoxidation). These reactions generate a variety of AGEs and cross-links (Fig. 5) (222, 260, 326). Some AGEs, such as *S*-carboxymethyl-cysteine (CMC), and *S*-carboxyethyl-cysteine (CEC), GOLD, MOLD, and DOLD (Fig. 5) may also result from amino acids (12) and lipids (109). MGO may react with arginine to form hydroimidizalones argpyrimidine and similar compounds (325).

DNA is susceptible to glycation, which produces several nucleotide-AGEs. Deoxyguanosine is highly susceptible to glycation, but other nucleotides can be modified in the presence of high concentration of dicarbonyl compounds (325). Moreover, glycating agents may induce the formation of cross-links in DNA (325).

Aminophospholipids can be targeted by glycating agents (glucose and carbonyl compounds), thereby forming adducts such as glycated-, carboxymethyl-, and carboxyethyl-phosphatidylethanolamine (249).

B. Defenses against glycation and AGEs

Several mechanisms have emerged, during evolution, to limit the accumulation of AGEs-modified molecules and

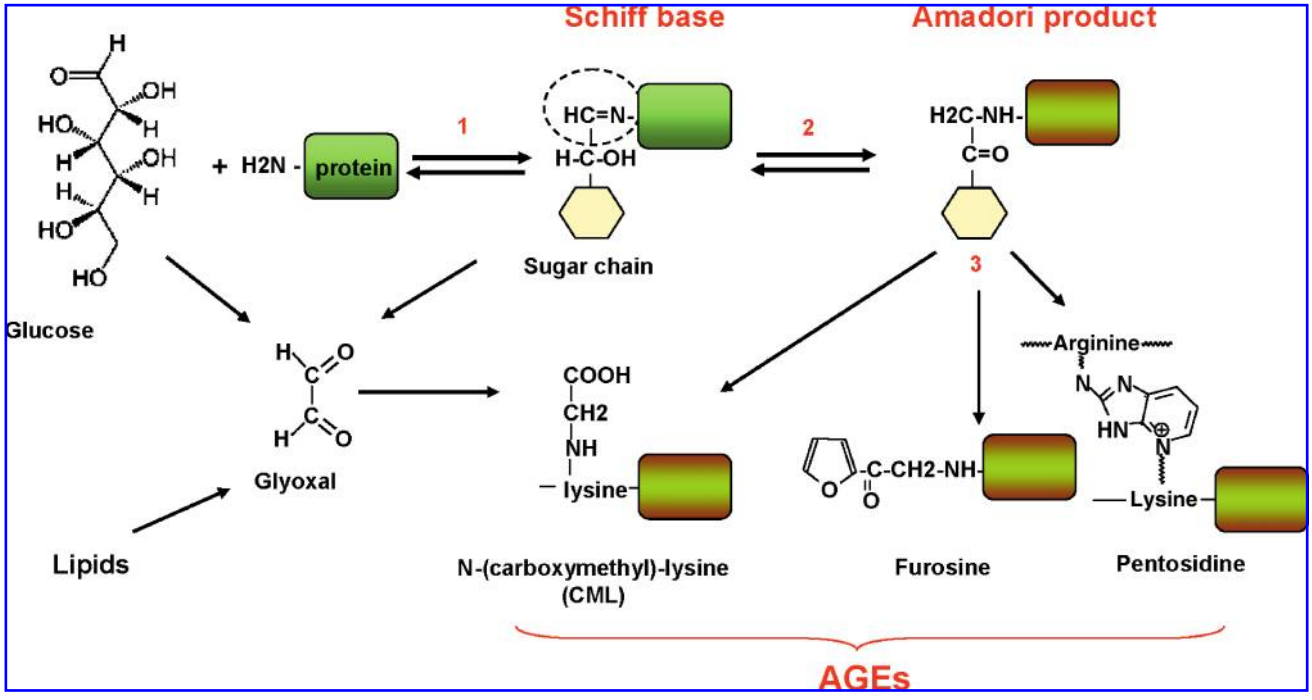

FIG. 4. The Maillard reaction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

Table 3. Levels of Selected Advanced Glycation Endproducts in Mammalian Tissues

Protein-tissue/specimen	*Levels*	*Method*	*Ref.*
Carboxymethyl-lysine (CML)			
Human serum			
Normal	73.2 ± 16.9 pmol/mg		
Hemodialysis F8[a]	308.8 ± 94.0 pmol/mg	HPLC	107, 108
Hemodialysis F80[a]	275.5 ± 79.1 pmol/mg		
Peritoneal dialysis	284.5 ± 98.2 pmol/mg		
Human LDL	0.034–0.06 mmol/mol lys		
LDLox (human)[b]	0.67–10.9 mmol/mol lys	SIMS-GC/MS	109
Skin			
Human	−1.5 mmol/mol lys (0–80 y)		
Normal	0.5–2.0 mmol/mol lys (20–80 y)	SIMS-GC/MS	89
Diabetes (type 1)			45
Rat	0.07–0.13 mmol/mol lys		
Normal	0.08–0.10 mmol/mol lys		
Dietary restriction	Increase from 0 to 30 mo[c]		
Lens crystallins	1.0–8.0 mmol/mol lys		
Human	Increase from 1 to 100 y[c]	SIMS-GC/MS	87
Implanted tendons, 28 d	7 ± 2 pmol/mg	HPLC	90
Normal rat	35 ± 7 pmol/mg		
Diabetic			
Heart mitochondria			
Rat	1,700 μmol/mol lys	SIM-GC/MS	247, 248
Pigeon	1,370 μmol/mol lys		
Rat (dietary restriction)	1,400 μmol/mol lys		
Skeletal muscle mitochondria	3,408 μmol/mol lys	SIM-GC/MS	28
Mouse			
Liver			
Rat, guinea pig, dog, pig, cow, horse	From 700 to 1,600 μmol/mol lys	SIM-GC/MS	249
Skeletal muscle			264
Rat	500 μmol/mol lys	SIM-GC/MS	
Pigeon	780 μmol/mol lys		
Carboxymethyl-ethanolamine (CME)			
Liver mitochondria			
Mouse, hamster, guinea pig, rabbit, sheep, pig, dog, cow, horse, human	From 0.5 to 1.5 mmol/mol ethanolamine	SIM-GC/MS	250
Carboxyethyl-lysine (CEL)			
Heart mitochondria			
Rat	575 μmol/mol lys	SIM-GC/MS	248
Rat (dietary restriction)	480 μmol/mol lys		
Skeletal muscle mitochondria			
Mouse	1,459 μmol/mol lys	SIM-GC/MS	28
Skeletal muscle			
Rat	250 μmol/mol lys	SIM-GC/MS	264
Pigeon	700 μmol/mol lys		

[a]F8 and F80 refer to dialysis membrane type, whereas F80 membrane indicates a larger pore size than F8.
[b]LDLs were oxidized with 5 μ*M* Cu(II) for 24 h.
[c]Age-related increase from the lowest to the highest value.

subsequent dysfunction. The main pathways implicated in AGEs metabolism are summarized in Fig. 6.

1. Glucose has low glycating activity. A first limitation of the Maillard reaction in animal organisms results from the chemical property of glucose, the main sugar, which is less reactive with amines than other sugars. This led to the postulate that glucose has been selected during evolution to minimize AGEs formation (38). The low reactivity of glucose with amines results from the high stability of its ring structure and, conversely, from the low level of the open configuration that is the most reactive form (because of the free carbonyl group). In comparison, galactose is 5 times more reactive with amines than glucose; fructose, 8 times; deoxyglucose, 25 times; and ribose, 100 times. Moreover, the level of blood glucose is maintained in a low range of concentrations (3–6 m*M*) by continuous and tightly regulated feedback mechanisms. Interestingly, the physiologic levels of other more-reactive sugars (fructose, ribose) and dicarbonyls (MGO) generated during the intermediary metabolism are maintained in a much lower (micromolar) range.

Table 4. Levels of Selected AGEs in Mammalian Tissues

Protein-tissue/specimen	*Levels*	*Method*	*Ref.*
Pentosidine			
Plasma protein			
Normoglycemia (human)	0.3–1.6 pmol/mg		
Diabetes (human)	0.5–4.8 pmol/mg	HPLC	239
End-stage renal disease (human)	5–55 pmol/mg		
Hemolysate		HPLC	239
Normoglycemia (human)	0.09–0.21 pmol/mg		
Diabetes (human)	0.05–0.22 pmol/mg		
End-stage renal disease (human)	0.2–1.5 pmol/mg		
Urine (total pentosidine)		HPLC	315
Normoglycemia (human)	4.2 ± 1.4 μmol/mol creat.		
Diabetes (human)	8.8 ± 4.3 μmol/mol creat.		
Skin collagen[a]			
Normoglycemia (rat, cow, pig)	0.3 pmol/mg	HPLC	297
Normoglycemia (monkey)	2–12 pmol/mg		
Normoglycemia (human)	0–100 pmol/mg		
Skin		HPLC	45
Normoglycemia (rat)	2–5 μmol/mol lys		
Dietary restriction (rat)	1.8–2.7 μmol/mol lys		
Cartilage		HPLC	331
Normoglycemia (human)	0–80 mmol/mol collagen		
Dura mater		HPLC	298
Normoglycemia (human)	25–225 pmol/mg		
Lens crystallins		HPLC	224
Normoglycemia (human)	1–1.5 pmol/mg		192
Diabetic (human)	1.5–2.5-fold increase		
Normoglycemia (dog)	1.2 pmol/mg		
Moderate diabetes (dog)	1.3–2.7 pmol/mg		
Severe diabetes (dog)	15.5 ± 16.1 pmol/mg		
Lung collagen			
Normoglycemia (rat)	40–120 pmol/mg collagen	HPLC	20
Pyrraline			
Serum albumin–rich fraction[b]			
Normoglycemia (human)	20–40 pmol/mg	ELISA	128
Diabetes (human)	30–60 pmol/mg		
Plasma[c]			
Normoglycemia (human)	194 ± 79 μM (*i.e.,* $\sim$2.5 nmol/mg)	ELISA	217
Diabetes (human)	627 ± 189 μM (*i.e.,* $\sim$8 nmol/mg)		
Plasma			
Normoglycemia (human)	12 ± 5 pmol/mg	HPLC	260
Diabetes (human)	21 ± 9 pmol/mg		
Lens crystallins			
Normoglycemia (human)	30 ± 10 pmol/mg	HPLC	223
Cataractous (human)	48 ± 12 pmol/mg		
Diabetes (human)	28 ± 15 pmol/mg		
Urine			
Normoglycemia (human)	1.2 μg pyrraline/mg creatinine	HPLC	261
Diabetes (human)	1.3 μg pyr/mg creat (range, 0.2–4)		

[a]Curvilinear increase with age found for all mammalian species examined.
[b]The discrepancy between the levels in serum albumin–rich fractions *versus* plasma proteins stems from the way the assay was calibrated.
[c]Plasma values are probably 100 times too high.

Another mechanism reduces the level of intracellular glucose and minimizes the carbonyl stress, by polymerizing excess glucose into glycogen, a nonreducing (thus nonglycating) polysaccharide.

2. The digestive barrier limits the absorption of diet-derived AGEs. Glycation of food is known to reduce the nutritional value of proteins. Only a few studies have investigated the digestion and the absorption of food-derived AGEs. Their absorption has been estimated at between 10 and 30% of ingested AGEs (169). This suggests that the digestive barrier limits the bioavailability of food-derived AGEs, but the small amount absorbed could participate in the carbonyl stress, mainly in the case of associated diseases, such as diabetes or nephropathy (169).

3. Cellular defense mechanisms. Several mechanisms tend to limit the level of cellular AGEs-modified proteins by scavenging and metabolizing carbonyl compounds.

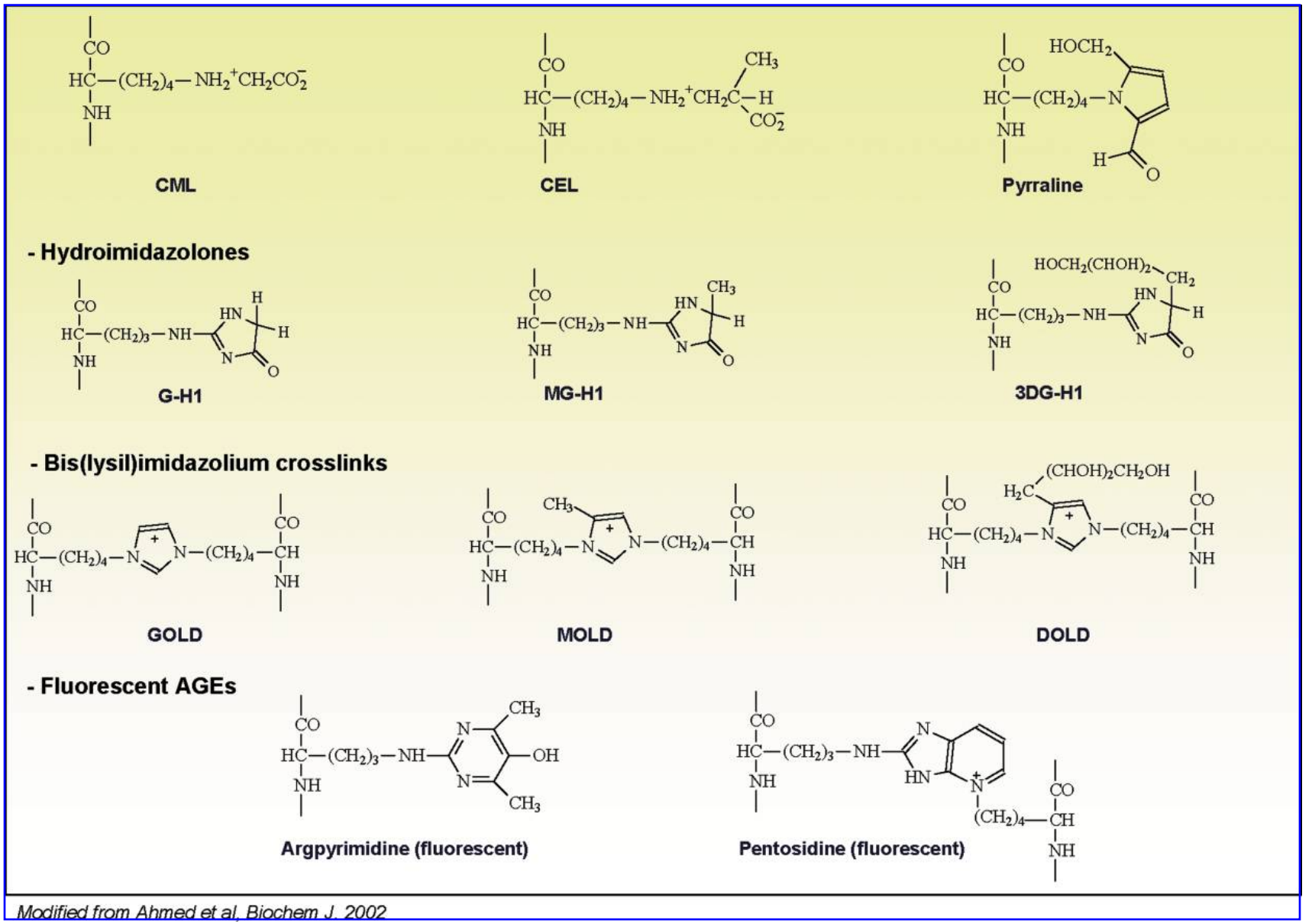

FIG. 5. Molecular structures of some AGEs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

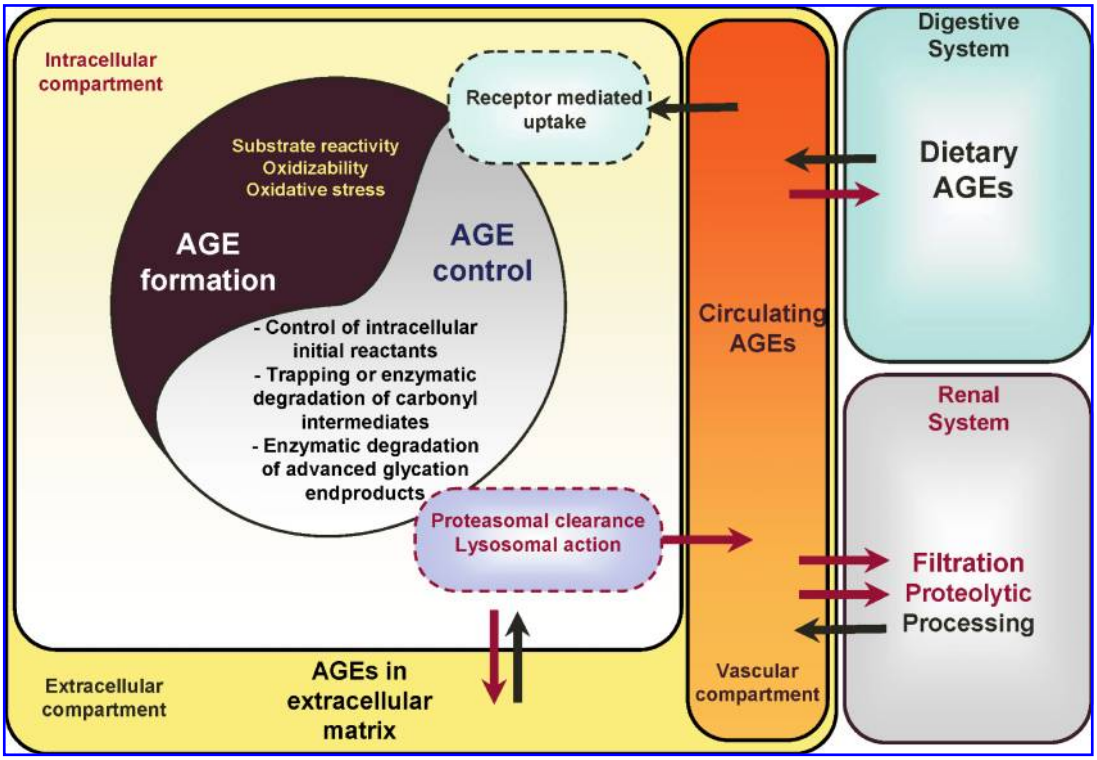

FIG. 6. AGEs metabolism and factors influencing AGEs homeostasis and their concentration in body compartments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

a. Low-molecular-weight agents

(1) Reduced glutathione. Glutathione in its reduced form (GSH) functions as a free radical scavenger, and as cofactor of glutathione peroxidase (GPx) and glutathione *S*-transferase (GST). GSH is a major endogenous antioxidant and carbonyl scavenger agent, which contributes to maintain the cellular redox status and protects cells from oxidative stress (176, 199). GSH depletion in diabetes is the consequence of ROS and carbonyl compounds generation, which react with GSH, thereby altering the redox equilibrium and leading finally to oxidative stress. Carbonyls react with GSH, both through a nonenzymatic mechanism, and through a GSH transferase (GST)-mediated mechanism. Finally, thiol- and histidine-containing dipeptides (including carnosine) can act as carbonyl-trapping agents (8).

(2) Carbon monoxide. Carbon monoxide (CO) is an endogenous gaseous factor produced during heme degradation by heme oxygenase-1. The vasorelaxant effect of CO implicates two mechanisms: (a) increased levels of cGMP, which inhibit inositol triphosphate generation and Ca^{2+} channel opening, thereby decreasing intracellular calcium $[Ca^{2+}]_i$ concentration; and (b) opening of high-conductance calcium-activated K^+ channels (KCa), which results in membrane hyperpolarization, inhibition of inositol triphosphate release, and decrease of Ca^{2+} level (339). In diabetic (STZ-treated) rats, the vasorelaxant effect of CO mediated by cGMP is maintained, whereas its regulatory effect on KCa-channel activity is reduced. This decrease in CO-vasorelaxant activity results from the glycation of KCa channel proteins (339).

(3) Bilirubin and biliverdin reductase. Bilirubin is produced throughout the catabolism of heme, which generates biliverdin that is reduced to bilirubin by biliverdin reductase (BVR). Bilirubin exhibits potent antioxidant activity (15). Bilirubin augments the rate of glucose transport and GLUT-1 expression and reverses the high glucose–induced down-regulation of the glucose-transport system in endothelial cells (65). Low serum bilirubin levels are associated with microalbuminuria and subclinical atherosclerosis in patients with type 2 diabetes (111). Likewise, BVR exhibits strong antioxidant activity and modulates the biologic function of IRS-1 and PI3K, through its binding with the p85 subunit of PI3 kinase (15). It is important to note that BVR is a leucine zipper–like DNA-binding protein, which can translocate into the nucleus and act as a transcription factor for activator protein 1 (AP-1)-regulated genes. Moreover, BVR could modulate ATF-2 and heme oxygenase-1 expression, suggesting its potential role in AP-1 and cAMP-regulated genes (99).

(4) Uric acid. Uric acid is the final oxidation product (in humans) of purine metabolism and is generated by xanthine oxidase from xanthine and hypoxanthine. Uric acid is a potent antioxidant in physiologic conditions (in humans, uric acid represents the half of the blood plasma antioxidant capacity) (111). However, hyperuricemia is also an important risk factor and even a risk marker for diabetes and its cardiovascular and renal complications. Elevated concentrations of uric acid become prooxidant in an oxidative environment, where uric acid functions as an "antioxidant/prooxidant urate redox shuttle." In the vascular wall, this contributes to enhance LDL oxidation and to promote accelerated atherosclerosis (129).

(5) Vitamins. Vitamins A (retinol), E (tocopherol), and C (L-ascorbic acid) are essential diet-derived nutrients for humans; they directly neutralize free radicals and ROS. They can interact through recycling processes, which regenerate their reduced forms. Vitamin E reacts directly with peroxyl and superoxide radicals and plays a key role in protecting membranes against lipid peroxidation. Although it is debated, the plasma levels of vitamins A, C, and E are not significantly altered in diabetes patients and in experimental animal models for diabetes (199). The retinol-binding protein 4, the plasma carrier of retinol, is elevated in insulin-resistant subjects and in type 2 diabetes (reviewed by Redondo and colleagues, ref. 275).

Mild plasma vitamin B_1 (thiamine) deficiency is observed in diabetes patients (275), resulting in an impairment of the reductive pentose phosphate pathway (PPP). The administration of thiamine and the prodrug benfotiamine restores a normal PPP function and inhibits the subsequent diabetes complications (322). Vitamin B_6 (pyridoxal-phosphate), exhibits carbonyl scavenger properties and so could neutralize AGEs and AGEs-linked diabetes complications (228).

b. Cellular antioxidant defenses

(1) Superoxide dismutase. Superoxide dismutases (SODs) catalyze the dismutation of $O_2^{\cdot -}$ into H_2O_2 and O_2. Three forms of SOD have been described in humans, depending on the metal cofactor; two SODs are Cu/Zn-dependent, one (SOD1) is located in the cytoplasm, and one (SOD3) is in the extracellular medium, whereas Mn-dependent SOD2 is localized in the mitochondria. The effect of hyperglycemia on SOD activity is cell and tissue dependent in STZ-treated mice. Cu/Zn-SOD could be inactivated by glycation under hyperglycemic conditions, particularly in red cells from diabetes patients (160). However, glycation does not induce major alterations of SOD activity, which can be normalized or even increased by antioxidants (probucol, vitamin E, vitamin C), by captopril, and DHEA, when administered to the animals at the beginning of the protocol (199). Interestingly, the mutation of *Cu/Zn SOD1* gene in familial amyotrophic lateral sclerosis (ALS), renders SOD1 highly susceptible to glycation and inactivates its activity, suggesting a role for SOD1 glycation in the oxidative stress associated with neuronal dysfunction in familial ALS (316).

(2) Catalase. Catalase is expressed mainly in peroxisomes, where it catalyzes the decomposition of H_2O_2 to water and O_2. Catalase is very effective in neutralizing high toxic H_2O_2 concentrations without changing the levels of low concentrations (which are vital for many physiologic processes, whereas high concentrations are toxic for mammalian cells). Chemically induced diabetes in STZ-treated animals results in increased catalase activity in heart, aorta, and brain, whereas it is decreased in liver and red blood cells. These modifications are normalized by co-treatment of the animals with captopril, probucol, or aminoguanidine. However, these molecules have no effect when the treatment is administered several weeks after the beginning of diabetic status (199).

(3) Glutathione peroxidase. Glutathione peroxidase-1 (GPx-1) is involved in the detoxification of H_2O_2 and lipid peroxides. It is ubiquitously expressed in cytosol and mitochondria. Mutations within the *GPx-1* gene are associated with increased atherosclerosis and diabetes risk (181). GPx-1 expression is altered in diabetes. It is increased in the liver, kidney, aorta, and blood, whereas this activity is decreased in heart and retina (199). Although GPx-1 may be increased in the pancreas as a compensatory mechanism in regard to oxidative stress, the insulin-producing beta cells exhibit very low intrinsic levels of antioxidant proteins and activities, particularly GPx-1, which renders these cells very vulnerable to ROS (278, 279). Mice double KO for GPx-1 and apoE, and are rendered diabetic by STZ treatment, exhibit accelerated diabetes-associated atherosclerosis, associated with an upregulation of proinflammatory and profibrotic pathways, thereby establishing GPx-1 as an important antiatherogenic therapeutic target in diabetes patients (181). However, some discrepancies exist on the protective role of GPx-1, because McClung (205) reported that overexpression of GPx-1 in mice results in hyperglycemia, hyperinsulinemia, and mild insulin resistance, this effect potentially resulting from an overquenching of ROS necessary to insulin signaling.

(4) Glutathione reductase and glutathione S-transferase. Glutathione reductase reduces the oxidized glutathione disulfide (GSSG) to the reduced GSH. STZ-treated diabetic animals exhibit reduced levels of glutathione reductase activity in retina and plasma, and increased activity in the heart (199).

Glutathione *S*-transferase (GST) is a cytosolic enzyme that catalyzes the conjugation of GSH to a variety of substrates, which allows it to neutralize peroxidized lipids, toxins, and xenobiotics. GST belongs to a family of multifunctional enzymes (301), and the human isoenzymes GSTA, GSTM, and GSTP play a role (mainly GSTA4-4) in the detoxification of a variety of carbonyl compounds.

(5) Heme oxygenase. Heme oxygenase catalyzes the degradation of heme, producing biliverdin, iron, and carbon monoxide (161). Two isoforms of heme-oxygenase have been described: heme-oxygenase-1, which is induced by oxidative stress, hypoxia, and cytokines; and heme-oxygenase-2, which is constitutively expressed. A third isoform, heme-oxygenase-3, is not apparently catalytically active, and its role is not known (161). Heme-oxygenase-1 is a key enzyme in cellular defenses, which protects the vasodilator function of endothelium, inhibits inflammation and apoptosis of endothelial cells, and contributes to vascular remodelling, neovascularization, and angiogenesis (4). The expression of heme-oxygenase-1 is upregulated by pharmacologic agents such as HMG-CoA reductase inhibitors, metalloporphyrins, or nitric oxide donors.

Heme-oxygenase-1 plays a major protective role in cardiovascular diseases and in diabetes complications (reviewed by Abraham and Kappas, ref. 4). When overexpressed in endothelial cells, heme-oxygenase-1 protects against apoptosis caused by various oxidative stress–inducing agents, including cytokines and hypoxia, and probably *via* a heme-oxygenase-1–mediated CO release (30). Heme-oxygenase-1 overexpression reverses the oxidative-mediated inhibition of EC proliferation induced by high-glucose conditions (4). Gene-transfer experiments of the human heme-oxygenase-1 in hyperglycemic rats decrease significantly ROS generation and oxidative stress, thereby preventing endothelial dam-

age (4). However, no major variations in heme-oxygenase-1 expression are observed in experimental diabetic animals (4).

ApoA1-mimetic peptides, such as L-4F, recently were developed and tested in animal models for atherosclerosis and diabetes. These peptides exert an antioxidant and antiapoptotic activity by decreasing the generation of $O_2^{\circ -}$ and increasing heme-oxygenase-1 activity in aorta and bone marrow, as well as the levels of CO and bilirubin and eNOS activity, thereby ameliorating the vascular function in diabetic animals (258). Moreover, L-4F improves insulin resistance in the ob/ob mouse model of diabetes, by increasing the activity of heme-oxygenase-1 and pAMPK, as well as the level of adiponectin and the phosphorylation of insulin receptor (257, 258).

(6) Thioredoxin. Thioredoxin is an oxidoreductase enzyme with a dithiol-disulfide active site. It catalyzes the reduction of proteins by thiol-disulfide exchange. Thioredoxins are ubiquitously expressed and maintain a reduced intracellular redox state in mammalian cells by the reduction of protein thiols (23). Thioredoxin plays a protective role in diabetes by controlling apoptosis as well as carbohydrate and lipid metabolism (186). Glucose loading reduces the level of thioredoxin in humans (211).

c. Cellular defenses against glycation and AGEs

(1) Amadoriases. Amadoriases are enzymes capable to metabolizing (deglycating) Amadori products. Two major classes of amadoriases exist in mammals (33, 76, 218).

1. Fructosamine oxidases, a subgroup of amadoriases, catalyze the oxidative deglycation of low-molecular-weight fructosamines (Amadori products), converting fructoselysine to lysine, H_2O_2, and glucosone.
2. Fructosamine kinase, another amadoriase subgroup, phosphorylates the Amadori compound at C3, producing fructosamine 3-phosphate residues, which are unstable, and decompose into 3-deoxyglucosone, amine, and phosphate (76). Fructosamine kinase–knockout mice exhibit elevated amounts of fructoselysine, as expected, but, surprisingly, have no phenotype (334), thus raising the question of the pathophysiologic importance of this deglycation pathway.

(2) Glyoxalase system. The glyoxalase system is composed of two GSH-dependent enzymes, glyoxalase I and glyoxalase II, which convert α-oxoaldehydes into α-hydroxy acids. Glyoxalase I acts on the hemithioacetal (formed by the nonenzymatic reaction between 2-oxoaldehyde and GSH) to form hydroxyacylglutathione derivatives, which are converted by glyoxalase II into α-hydroxyacids, whereas GSH is regenerated (206). The glyoxalase system metabolizes reactive α-oxoaldehydes, such as MGO, GO, hydroxypyruvaldehyde, and 4,5-doxovalerate, thereby preventing the carbonyl stress and its deleterious effects. Interestingly, overexpression of glyoxalase-I completely prevents AGEs formation in cells grown in high-glucose medium (302). Finally, it may be noted that oxidative stress depletes GSH (NADPH used to reduce GSSG), thereby decreasing the activity of NADPH-dependent enzymes, such as glyoxalase-I and aldehyde reductases (342).

d. Proteolytic degradation by proteasome. Damaged cellular proteins are degraded by several proteolytic systems, including the proteasome (119). Proteasome is a large proteolytic complex that hydrolyzes tagged (ubiquitinated) as well as misfolded and oxidized proteins. In contrast, proteins extensively modified by carbonyl compounds are poorly degraded by the proteasome, inhibit the proteasomal activity, and tend to form protein aggregates (123).

4. Role of the kidney and liver in the excretion of AGEs. Circulating AGEs consist of AGEs-modified plasma proteins, AGEs–low molecular weight compounds (for instance, AGEs peptides), and "AGEs free adducts" (*e.g.*, AGEs single amino acids) originating from exogenous AGEs. The latter represent the most abundant form of AGEs in the plasma of both healthy and diabetic subjects and are either generated by direct modification of circulating amino acids or released during the degradation of AGEs-modified proteins and other macromolecules (5, 325). Circulating AGEs can be eliminated by either renal or hepatic clearance.

In the kidney, AGEs-adducts to amino acids and peptides are filtered into the glomeruli. AGEs-amino acids are eliminated in urine, whereas AGEs-peptides are partly eliminated and partly reabsorbed in the proximal tubule, to be degraded in the lysosomes and produce AGEs-amino acids, the latter being then excreted in urine (5). The predominant plasma AGEs (*e.g.*, CML and CEL) have a high renal clearance. Renal failure leads to the retention of AGEs, which are toxic and aggravate renal lesions.

The hepatic uptake of AGEs-modified serum albumin is mediated by scavenger receptors, but the hepatic clearance of protein or free AGEs remains controversial (126, 295).

C. Molecular and cellular consequences of glycation and AGEs formation

1. Glycation of circulating proteins. The glycation of serum proteins is characterized by the formation of Amadori glucose adducts on free amino groups. The biologic signaling of glycated proteins is mediated through receptors different from receptors for AGEs (RAGE), which display AGEs signaling (68). However, similarities exist in the cellular signaling mediated by both Amadori and AGEs.

a. Hemoglobin. The glycation of hemoglobin through the Maillard reaction leads to the formation of hemoglobin A1c (HbA1c). It results from the formation of an adduct between glucose and the N-terminal valine amino group of the β chain of hemoglobin. The determination of HbA1c in diabetes patients is a reliable indicator for monitoring glycemic control in diabetes patients (342). HbA1c could participate to increase the chronic renal failure (299).

b. Insulin. Hyperglycemia induces the glycation of insulin in pancreatic β cells (3). The site of glycation in humans has been identified as the NH_2-terminal Phe^1 of the insulin B-chain (238). Glycated insulin is unable to regulate glucose homeostasis *in vivo* and to stimulate glucose transport and adipose tissue lipogenesis (3).

c. Serum albumin. Glycated serum albumin represents the largest portion of the circulating glycated proteins (~80% of

total protein glycation). The predominant Amadori modifications are observed on lys-525, lys-439, lys-281, and lys-199 (68). The circulating level of glycated serum albumin reflects the glycemic status in the previous weeks (half-life of normal serum albumin is ~20 days, whereas the clearance of glycated albumin is faster in rabbit, with a half-life ~70% of the control) (156).

Glycated albumin exhibits a large range of biologic properties that have been extensively studied by Cohen's group (68). In brief, these biologic properties are comparable to AGEs, but do not implicate RAGE, and are not blocked by RAGE inhibition (68). The expression of Amadori receptors has been reported in murine endothelial cells and glomerular mesangial cells. Calnexin could also bind glycated albumin in murine mesangial cells (344). Glycated albumin is transported across the glomerular filtration barrier and taken up by glomerular mesangial and epithelial cells; thus, its relevance in diabetic nephropathy is well established (68).

Glycated albumin generates a proinflammatory signaling resulting from oxidative stress and NF-κB activation (68, 78). NADPH oxidase and ROS-dependent signaling activate PKC, ERK, JNK, IκB kinase, NF-κB, and AP-1 transcription factor (133). This results in endothelial dysfunction characterized by (a) a reduced expression of constitutive eNOS (51) and an enhanced expression and activation of iNOS (11); and (b) an overexpression of cell-surface adhesion molecules leading to mononuclear cell adhesion and endothelium activation (78). In glomerular endothelial cells, glycated albumin stimulates the synthesis of fibronectin and collagen IV (57), thus participating to thicken the glomerular basement in diabetes (69).

Likewise, TGF-β is generated from murine macrophages exposed to glycated albumin, *via* ROS, NF-κB, and ERK1/2 activation (68, 150). Glycated albumin increases the expression of VEGF in peritoneal mesothelial cells, which could increase vascular permeability, vasodilation, and neoangiogenesis in patients undergoing peritoneal dialysis (197).

Glomerular nephropathy occurring in diabetes complications is ameliorated by monoclonal antibodies directed against glycated albumin (66). These antibodies lower the level of glycated serum albumin in db/db mice and reduce the proteinuria and the accumulation of extracellular matrix proteins and mesangial expansion. Interestingly, pharmacologic inhibitors of glycation, such as 23CPPA, inhibit the overexpression of VEGF and TGF-β and the subsequent synthesis of fibronectin and collagen IV implicated in mesangial matrix expansion (67).

d. Lipoproteins

(1) LDL and VLDL. The level of glycated LDL and VLDL is significantly increased in diabetes patients (318) and is always higher than the level of circulating oxidized LDL (329).

Glycation of VLDL impairs their degradation by lipoprotein lipase (LPL), which contributes to increase the plasmatic accumulation, hypertriglyceridemia, and impaired clearance of these lipoproteins. Glycated VLDLs are implicated in diabetic nephropathy (196). Glycation of apoB occurs on lysine residues (309) and phosphatidylethanolamine (274). Lipoprotein (a) (LPa) and small dense LDL, which are increased in diabetes (171), are most sensitive to the glycation process than LDL of lower density (360). Glycated LDLs are implicated in accelerated atherosclerosis (360).

(2) Increased sensitivity to oxidation. Glycated LDLs are prone to oxidation, which may occur during glycation, *via* the generation of free radicals from glucose and Amadori products when forming adducts on the protein (50). In addition, the inflammatory signaling (oxidative stress) elicited by glycoxidized LDL contributes to increase the oxidation of glycated LDL (50). The oxidation process is blocked by antioxidants (although antioxidants do not inhibit LDL glycation) (182).

(3) Foam cell formation. Glycoxidized LDLs are present in atherosclerotic lesions from diabetes patients and are localized in macrophages (148). Glycated and glycoxidized LDLs are taken up by macrophages through scavenger receptors, including SR-A, CD36, SR-BI, and LOX-1 (139). The expression of scavenger receptors is stimulated by glycoxidized LDL (175), LOX-1 (304).

(a) Glycated LDL-mediated inflammatory signaling. Glycated LDLs (like oxidized LDL), and LDLs from diabetes patients, trigger an intracellular oxidative stress characterized by ROS generation and a decrease in GSH content. However, glycated LDLs do not alter the expression levels of antioxidant enzymes, SOD, GPX-1, and catalase activities in vascular cells (362).

Glycated LDLs trigger a progressive impairment of endothelium-dependent relaxation because of the release of superoxide anion, and its condensation with NO to form peroxinitrite ($ONOO^-$) (114, 265). Likewise, glycated LDLs impair NO generation *via* a decrease in arginine uptake (265) and a decrease in eNOS expression *via* mechanisms implicating oxidative stress, intracellular Ca^{2+} increase, and Ca^{2+}-dependent calpain activation (82). Glycoxidized LDLs, but not glycated LDLs, strongly alter the generation of PGI_2, which contributes to trigger endothelial dysfunction (221).

Glycated LDLs isolated from diabetic rats upregulate the expression of CC chemokine receptor 2 (CCR2) and of MCP-1 and MCP-1 receptor (149, 308), implicated in macrophage chemotaxis (97). This dual effect on both CCR2 and MCP-1 could explain in part how glycated LDLs and AGEs-LDLs promote the migration and the accumulation of monocytes/macrophages in diabetic atherosclerotic lesions (149). Glycated LDLs isolated from diabetes patients inhibit the cell-cycle progression of HUVECs, through the formation of a prolactin-inducible element (PIE)-binding complex containing the transcription factor STAT5, and the cell-cycle inhibitor p21(waf) (29). In human mesangial cells, glycated LDLs upregulate the expression of IL6, CD40, and the release of fibronectin and laminin, thus participating in the alteration of the basement membrane (286).

(b) Mitogenic and apoptotic effects. Low concentrations of glycated LDLs are mitogenic for aortic smooth muscle cells, through mechanisms implicating PKC, phospholipase C, and the MEK/ERK1/2 pathway (60). Conversely, higher concentrations are antiproliferative and induce apoptosis by activating the JNK apoptotic pathway (286) and the proapoptotic Bak and caspase-3 (13).

(c) Alteration of platelet function and procoagulant activity. Glycated LDLs increase the reactivity of platelets to aggregating agents, particularly ADP, thrombin, and collagen (97, 368). Moreover, glycated LDLs progressively inhibit the platelet membrane Na^+/K^+-ATPase activity and increase the

Ca^{2+}-ATPase activity and the intracellular Ca^{2+} concentration (97). Glycated LDLs are prothrombogenic, as they decrease the synthesis of tissue plasminogen activator (tPA) and enhance the expression of PAI-1 (193), perhaps mediated by an increased expression of the heat-shock factor 1 (HSF1), which binds the PAI-1 promoter (363).

(d) HDL. The glycation of apoA-1 results in a progressive decrease in its antiatherogenic properties, particularly its ability to proceed to normal cholesterol reverse transport to the liver (168, 171). Modification of apoA1 by MGO induces conformational changes that render HDLs unable to activate lecithin-cholesterol acyltransferase (LCAT), a key enzyme implicated in the reverse transport of cholesterol process (237). Similarly, glycated HDL-3 fails to activate cholesterol ester transfer by cholesteryl ester transfer protein (CETP) (179), and to stabilize the ATP-binding cassette transporter A1 (ABCA1), an atheroprotective cell protein involved in the transport of cholesterol from cells to HDLs (135). Glycation of HDLs is correlated with a decrease in their antioxidant properties and the loss of paraoxonase (PON1) activity (96, 200). This lower antioxidant capacity impairs the antiinflammatory properties of HDLs, which become unable to counteract oxidized and glycoxidized LDL-mediated signaling [for instance, the expression of adhesion molecules in monocytes and macrophages (135)], or the inhibition of endothelium-dependent vasodilation (256). When HDLs are oxidized and exposed to high glucose concentration, caspase 3 and 9 are activated in cultured endothelial cells that undergo apoptosis (203), but no evidence has been reported for a proapoptotic effect of glycated HDLs from diabetes patients. It is important to note that glycation may directly affect ABCA1 (252) and SR-B1. SR-B1 is a known receptor for HDLs, but may serve also as receptor for AGEs, which affects both selective HDL-cholesteryl ester uptake and cholesterol efflux from cells to HDLs (241).

2. Molecular and cellular effects of AGEs modification. AGEs interact with AGEs-specific receptors, which induces oxidative stress and proinflammatory signaling responses. Moreover, AGEs can directly react with cellular and tissue proteins, thereby modifying their structure and properties. Both RAGE-dependent and RAGE-independent effects of AGEs are involved in the pathogenesis of diabetes complications.

a. RAGE- (and related receptors)-dependent effects. A group of proteins located at the plasma membrane are able to bind AGEs. This includes the classic RAGE (receptors for AGEs) and other proteins [AGEs-receptors OST-48 (AGE-R1), 80K-H (AGE-R2), galectin-3 (AGE-R3) (321), and scavenger receptors class A type I and II, scavenger receptors class B (CD-36), and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1)] (Fig. 7).

(1) RAGE. RAGE represents the most important system implicated in AGEs binding. Its interaction with the ligands triggers proinflammatory signaling, implicating oxidative stress and NF-κB. The implication of RAGE and AGE/RAGE binding in hyperglycemia and diabetes complications has been most extensively studied by the group of Schmidt [see review by Wautier and Schmidt (342) and by Yan *et al.* (357)].

(a) Structure of RAGE. First characterized by Neeper *et al.* (231), RAGE is single 35-kDa transmembrane receptors that belong to the immunoglobulin superfamily of cell-surface molecules (271, 290, 310), which also includes CD36, scavenger receptor B1 (SR-B1), and low-density lipoprotein-related receptor (LRP) (310). RAGE is formed of 394 amino acids organized in a large extracellular region of 332 amino acids, with three immunoglobulin-like domains, (one V-type and two C-type immunoglobulin domains) (71), a transmembrane region characterized by a single hydrophobic spanning domain, and a small (40 amino acids) and highly charged cytosolic region at the carboxy terminus. Ligands interact with the V-domain of the receptor (137), whereas the cytosolic region is implicated in RAGE signaling.

(b) RAGE gene and polymorphisms. The human RAGE gene exhibits a total length of 1,500 bp, including the promoter region (141). It is encoded on chromosome 6 within the major histocompatibility complex (MHC) class III region (141, 289). At least 30 polymorphisms have been reported so far, with

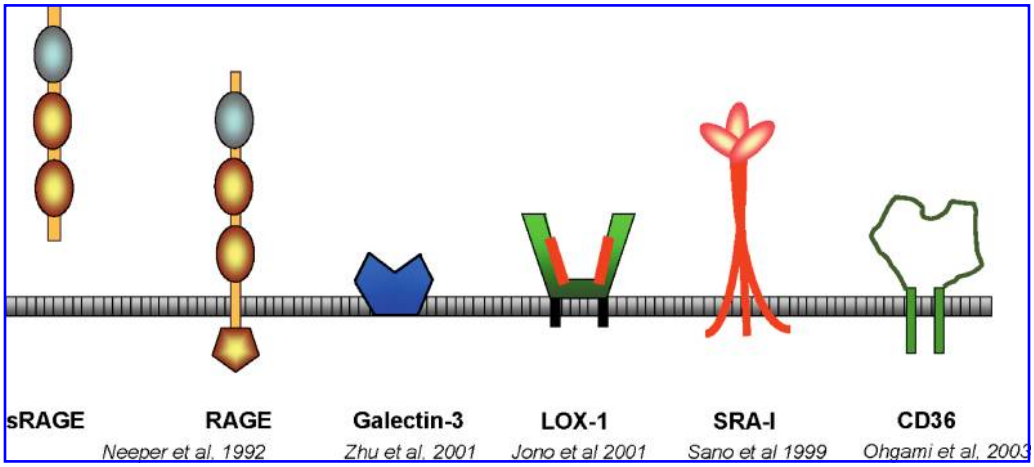

FIG. 7. Schematic representation of some receptors for AGEs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

consequences on RAGE expression and function (80). The single-nucleotide polymorphism (SNP) rs3134945 is involved in the development of insulin resistance and diabetes (142), whereas polymorphisms G82S, 1704G/T, 2184A/G, and 2245G/A are correlated with alterations of the antioxidant status in type 2 diabetes (158). The 374T/A polymorphism in RAGE gene promoter is associated with macrovascular complications in type 1 diabetes (94). However, most polymorphisms are unrelated to the pathophysiology of diabetes.

(c) Alternative splicing. At least six isoforms of RAGE could result from alternative splicing of a single RAGE gene (80). This includes the full-length transmembrane receptor, the dominant-negative RAGE (dnRAGE), and soluble RAGE (sRAGE). Most isoforms express the V- and C-type domains, whereas soluble RAGE (sRAGE) and endogenous secretory RAGE (esRAGE) lack the transmembrane domain and are secreted from cells.

(d) RAGE expression. RAGE is ubiquitously expressed, and their expression is upregulated by the ligands, in particular, in atherosclerotic lesions, glomerular tissues, and retina of diabetes subjects (12, 271). sRAGE and esRAGE constitute suitable biomarkers for the follow-up of diabetes vascular and renal complications (227, 235), and for the early-stages of carotid atherosclerosis in type 1 diabetes (159). Both esRAGE and sRAGE reduce the deleterious effects of AGEs and represent a new therapeutic target for diabetes vascular complications (235).

(e) Ligand specificity

(i) AGEs. RAGE is multiligand receptors that recognize several types of AGEs and non-AGEs ligands. RAGE interacts with CML (162), CEL, pentosidine, pyrraline, and 3 DG-hydroimidazolones (154, 321). RAGE affinity is higher for ribose AGEs than for glucose and fructose AGEs (333). RAGE interacts with MGO (22) and with toxic AGEs (TAGEs) deriving from glyceraldehyde (287), but not with Amadori–glucose adducts (68).

(ii) S100 calgranulins. Among the non-AGEs ligands, RAGE bind S100/calgranulins, which are a family of small calcium-binding proteins associated with inflammation (131). The precise biochemical and molecular mechanisms that regulate the expression of S100s are only partly understood. Among the different calgranulins, S100B could be a marker of brain damage and malignant melanoma (164, 283). S100A12, also called EN-RAGE for "extracellular newly identified receptor for advanced glycation end products binding protein," may contribute to the development of atherosclerosis and cancers (220).]

(iii) High-mobility-group box protein-1. High-mobility-group box protein 1 (HMGB1), also called amphoterin, is a highly conserved member of the HMG (high-mobility group)-1 family of DNA-binding proteins. Extracellular HMGB1 regulates cell motility and migration through its binding to receptors such as TLR4, TLR2, and RAGE (273).

(iv) Amyloid-ß peptide and ß-sheet fibrils. Amyloid-ß peptide and β-sheet fibrils are additional ligands for RAGE and induce oxidative stress, inflammation, and amyloidosis (58). RAGE bind the islet amyloid polypeptide (IAPP), which is implicated in the development of islet amyloidosis, and results in a progressive destruction of insulin-producing β cells and islet dysfunction (72, 354). Excess production and secretion of IAPP, along with insulin, is a major consequence of hyperglycemia, but also is thought to cause diabetes (64). The deleterious effect of amyloidosis in the pathogenesis of islet lesion in type 2 diabetes could result from chemical modifications or glycation of IAPP or both (194). It is important to note that hyperglycemia contributes to the glycation of tau and of amyloid-β protein, which markedly enhances its aggregation. This may have implications in the pathophysiology of chronic neurodegenerative diseases, in particular Alzheimer's disease, by amplifying the neurotoxic effect of amyloid-β peptide on microglia, blood–brain barrier (BBB), and neurons.

(2) AGEs receptors and scavenger receptors. The other receptors able to bind AGEs are AGE-R1, 2, and 3; scavenger receptors class A and class B; and lectin-like oxidized low-density-lipoprotein receptor-1.

(a) AGE-R1 or OST-48 (oligosaccharyl transferase-4). AGE-R1, a 60-kDa protein, is homologous to Ost-48, a component of the oligosaccharyltransferase complex located in the rough endoplasmic reticulum. AGE-R1 negatively regulates the inflammatory response of AGEs by inhibiting oxidative stress and NF-κB activation, *via* EGFR and Shc/Grb2/Ras (41). The expression of AGE-R1, like other anti-AGE defenses, is decreased in severe diabetes complications (191).

(b) AGE-R2 (80K-H or PRKCSH). AGE-R2 is a protein kinase C and tyrosine kinase substrate (117, 182). It is a weak AGE receptor, because the binding of AGEs-modified proteins is not inhibited by anti-80K-H antibodies in most cells (146). It is involved in FGFR3 signal transduction to MAP kinase and may play a role in the signal transduction elicited by the AGEs-receptor complex.

(c) AGE-R3 or galectin-3. Galectin-3 (AGE-R3) binds AGEs, but also other ligands, such as acetylated and oxidized LDLs; thus, it behaves as a scavenger receptor (366). The expression of galectin-3 is low at the basal state, and it increases in diabetes conditions (267). Galectin-3 could associate with other AGEs receptors, as a molecular "AGEs-receptor complex" (336).

Galectin-3–deficient mice develop accelerated diabetic glomerulopathy and nephropathy, associated with an increased renal accumulation of AGEs, thereby indicating a protective mechanism for galectin-3 against AGEs-induced tissue injury, as opposed to RAGE (146).

(d) Scavenger receptors

(i) CD36. CD36 is a member of the class B scavenger-receptor family of cell-surface proteins, which binds many ligands including oxidized LDL and phospholipids (233). It is upregulated by AGEs (151) and binds AGE-modified proteins in macrophages (241). However, its implication as an AGEs receptor is debated, because Nakajou *et al.* (226) reported that the uptake of AGEs-modified proteins such as glycolalde-

hyde-modified BSA (GA-BSA) and MGO-modified BSA is not inhibited by neutralizing anti-CD36 antibodies.

(ii) MSR-A. Macrophage-scavenger receptors type-I and type-II class-A (MSR-A) are expressed by macrophages and play a major role in the accumulation of cholesterol during atherogenesis (32). MSR-A can bind many ligands, including AGEs proteins, and could play a role in AGEs uptake in the early stages of atherogenesis, whereas RAGE should be prevalent in the advanced lesions (285). Peritoneal macrophages isolated from MSR-A–knockout mice exhibit reduced uptake of AGE-BSA, when compared with controls (314).

(iii) SR-B1. SR-B1 belongs to the CD36 class B family and is expressed in vascular cells, where it is involved in the "selective cholesterol uptake" of HDL. SR-B1 may recognize modified (acetylated) LDL and AGEs-modified proteins *via* a common ligand-binding site different from that of HDL (241). However, the binding of AGEs proteins on SR-B1 hinders the binding and selective cholesterol uptake of HDL (241).

(iv) LOX-1. Lectin-like oxidized LDL receptor-1 (LOX-1) belongs to the class E family of scavenger receptors and recognizes oxidized LDL and AGEs (153). AGEs increase LOX-1 expression in diabetic rats (57).

(v) Other AGEs-binding proteins. FEEL-1 (fasciclin EGF-like, laminin-type EGF-like, and link domain–containing scavenger receptor-1) and FEEL-2 are endocytic receptors for AGEs (319). Stabilin-2 (but not the homologous stabilin-1) is able to bind and internalize AGEs-modified BSA and formaldehyde-treated BSA (125).

(3) AGE/RAGE-dependent cellular effects

(a) NF-κB activation and inflammatory signaling. The binding of RAGE ligands (AGEs, S100/calgranulins, HMGB-1, amyloid-β peptides, and β-sheet fibrils) to RAGE, generates an oxidative stress-dependent activation of NF-κB (132) (Fig. 8) that is supported by the inhibitory effect of antioxidants such as *N*-acetyl-L-cysteine (359), vitamins C and E, and polyphenols (140). The AGE/RAGE-induced oxidative stress implicates mitochondrial-mediated ROS generation and NADPH oxidase activation (184, 343). Macrophages isolated from diabetes patients exhibit increased NADPH oxidase activity and $O_2^{\cdot -}$ generation, increased respiratory burst, and phagocytic activity, which are reversed by RAGE inhibitors (81).The mechanism implicated in NADPH oxidase activation involves a protein kinase C (PKC)-β_2-dependent translocation of the $p47^{phox}$ NADPH oxidase subunit, inhibited by PKC inhibitors (184). A premature translocation of $p47^{phox}$ to the plasma membrane and its association with $p22^{phox}$ is also reported in resting neutrophils isolated from diabetes patients, and in HL-60 grown in high-glucose concentrations (25 m*M*) (244). Interestingly, the p22 subunit of NADPH oxidase is polymorphic, and the association of its C242T variant with the G1704T polymorphism of RAGE is correlated to the pathogenesis of diabetic nephropathy in type 1 and type 2 diabetes (202).

As summarized by Yan *et al.* (356), the mechanism leading to NF-κB activation implicates a cascade of ROS-mediated signaling pathways, which includes p21ras, ERK½ (p44/ p42), p38 and SAPK/JNK, rho GTPases, src, PI-3 kinase, and the JAK/STAT pathway (132, 356). However, the precise molecular mechanisms linking RAGE and NF-κB activation are still largely hypothetical. A role for the Toll-like receptor (TLR) system was recently reported, which implicates interactions between the interleukin 1 (IL-1)-receptor cytosolic domain (TIR) of TLRs and the RAGE cytosolic tail, followed by an oligomerization with intracellular TIR-containing adaptor proteins. These interactions trigger the formation of the IKK signalosome and NF-κB activation (184). However, direct interactions between RAGE and the signaling-cascade effectors leading to NF-κB activation are possible (184).

Most biologic effects resulting from AGE/RAGE signaling depend on ROS generation and NF-κB activation (Fig. 9).

(b) Arachidonic acid cascade. AGEs induce the expression of cyclooxygenase-2 (COX-2) (mRNAs and protein), and the production of prostaglandin E_2 (PGE_2) in macrophages, circulating monocytes, and chondrocytes (300). This effect is inhibited by anti-RAGE antibodies, by pharmacologic inhibitors of NF-κB, PKC, ERKs, and p38 MAPKs, and by antioxidants (300). Likewise, the interaction of HMGB-1 and RAGE mediates the expression of COX-2 and the proapoptotic proteins Bad and Bax, which participate in chronic intestinal inflammation and enterocyte cell death (361).

(c) Nitric oxide synthase. AGEs-modified proteins reduce eNOS expression by increasing mRNA degradation and inhibit eNOS activity by suppressing eNOS phosphorylation on serine (347). In contrast, AGEs increase iNOS and NADPH oxidase expression *via* a p38MAPK and NF-κB–mediated mechanism, thus triggering oxidative stress and $ONOO^-$ generation (54, 165).

(d) Endothelium activation. The decrease in NO bioavailability and NF-κB activation promotes an inflammatory activation of the endothelium characterized by the expression of selectins, VCAM-1, and ICAM-1 adhesion molecules, and the adhesion of leukocytes and monocytes (337) and of MCP-1 (149). Moreover, AGE/RAGE interactions promote the expression of endothelin-1 (270). Telmisartan, an angiotensin II type 1 blocker, inhibits the generation of $O_2^{\cdot -}$ and the expression of MCP-1, ICAM-1, and VEGF, *via* a peroxisome proliferator–activated receptor-γ (PPAR-γ)-mediated downregulation of RAGE (201, 350).

(e) Cell proliferation. The effect of RAGE signaling on cell proliferation is controversial. The binding of AGEs to RAGE triggers the activation of a mitogenic cascade implicating PKC, p21(ras), ERK1/2, and the AP-1 transcription factor (130, 338). Balloon injury in the carotid artery of diabetic Zucker rats stimulates SMC proliferation and neointimal formation, by triggering a strong increase in RAGE expression in diabetic animals, which is reversed by sRAGE (365). Conversely, RAGE signaling activates the JAK/STAT pathway and the expression of TGFβ-1, which decreases cell proliferation (130). The inhibitory effect of AGE/RAGE on proliferation could involve an inhibition of Akt (16).

(f) Angiogenesis. AGE/RAGE interactions participate with the impaired angiogenesis observed in diabetes patients. This was demonstrated in an *in vitro* model of angiogenesis

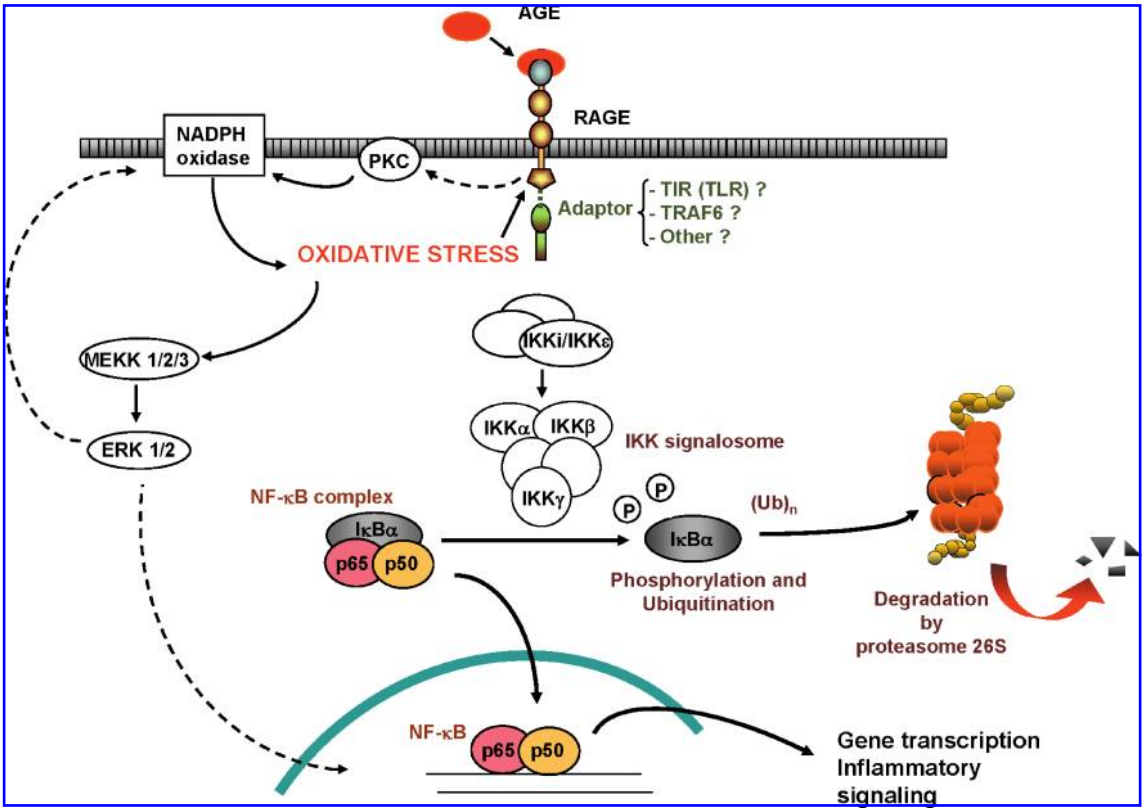

FIG. 8. AGE/RAGE interactions trigger oxidative stress and NF-κB activation. The binding of AGEs on RAGE results in the activation of NADPH oxidase through a translocation of regulatory subunits to the plasma membrane, which induces the generation of ROS ($O_2^{\cdot -}$ and H_2O_2). ROS activate a signaling cascade leading to the phosphorylation of I-κB (the cytosolic inhibitor of NF-κB) and its degradation by the proteasome. Free NF-κB may translocate into the nucleus, where it induces the transcription of proinflammatory genes. The molecular mechanisms by which ROS trigger NF-κB remain largely hypothetical but could implicate interactions between the RAGE cytosolic tail and adaptors from other receptors, such as the interleukin 1 (IL-1) receptor cytosolic domain (TIR) of the Toll-like receptor (TLR) system, which activates a signaling cascade leading to the formation of the IKK signalosome and NF-κB activation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

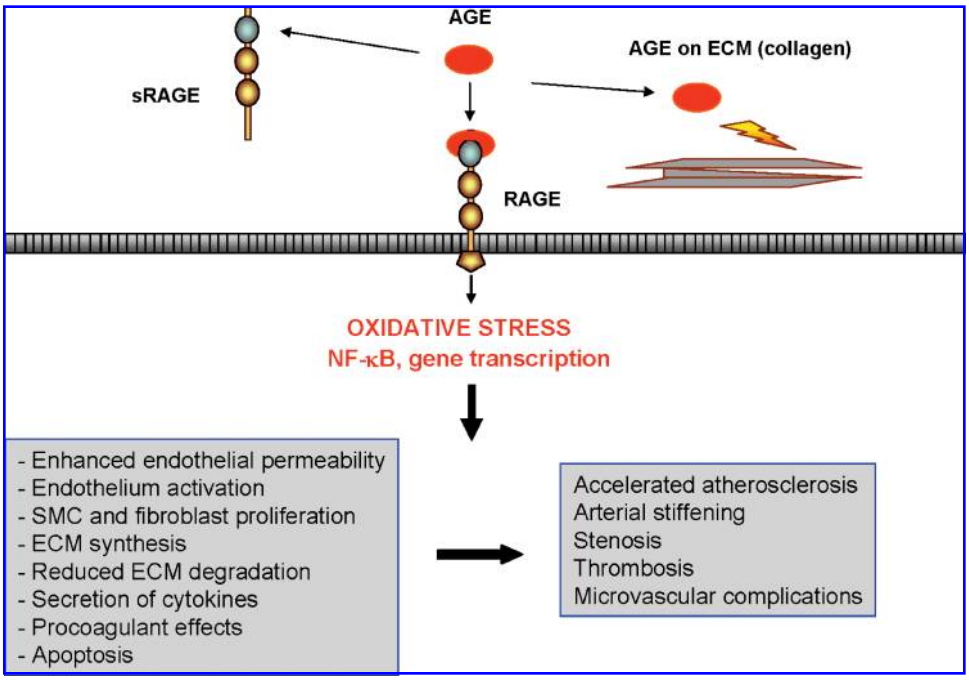

FIG. 9. Biologic effects resulting from AGE/RAGE interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

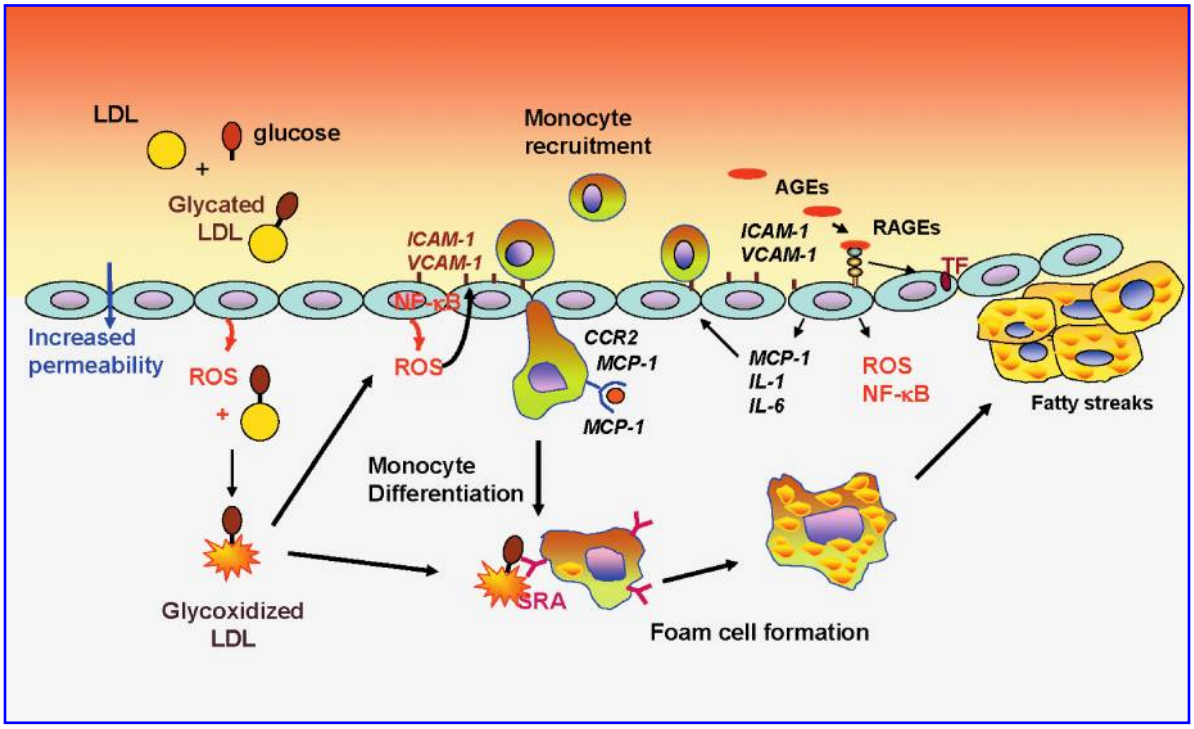

FIG. 10. Mechanism of accelerated atherosclerosis in diabetes. Hyperglycemia leads to the formation of circulating AGEs, which interact with RAGE expressed in endothelial cells. AGE/RAGE interactions trigger oxidative stress and NF-κB activation, which generates inflammatory signaling, endothelium dysfunction, and increased endothelium permeability. Increased levels of circulating LDLs become glycated in the hyperglycemic environment, transfiltrate the endothelial barrier, and accumulate in the intima, where they undergo rapid oxidation. Glycoxidated LDLs are taken up by macrophages by the scavenger-receptor pathway, which results in the accumulation of foam cells and fatty streaks. Glycoxidated LDLs are prothrombotic and proapoptotic and more generally behave as highly bioreactive cytotoxins that contribute to aggravating (like AGE/RAGE) the general proinflammatory environment of the atherosclerotic lesions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

showing that endothelial cell invasion into collagen gel is reduced when collagen is glycated (172). The inhibitory effect of AGEs on angiogenesis could result from a decreased degradation of AGEs-modified ECM components in diabetic mice, despite an increased expression of MMP-2, MMP-3, and MMP-13. AGEs-modified serum albumin inhibits the migration of endothelial progenitor cells (EPCs) by upregulating RAGE expression in these cells, which impairs their recruitment to regions of angiogenesis and leads to their apoptosis (313). It is important to note that the angiogenesis impairment reported in diabetes may also result from the glycation of the fibroblast growth factor 2 (FGF2). For instance, FGF2 glycated *in vitro* exhibits a lower angiogenic and mitogenic effect when injected into normoglycemic mice (88). However, the inhibitory effect of AGEs on angiogenesis is controversial, because AGEs may stimulate endothelial cell proliferation and tubes formation, *via* an upregulation of VEGF (242, 351).

(g) Cytokines, growth factors, and chemokines secretion. AGEs-modified proteins (AGE-LDL, AGE-albumin) stimulate the generation of inflammatory cytokines (TNF-α, interleukin-1, interleukin-6), growth factors (TGF-β1, IGF-1), metalloproteases (MMP-1, -2, -3, and -13), chemokines, and other bioactive molecules (337, 342). These agents amplify the proinflammatory response mediated by AGE/RAGE interactions and modulate cell proliferation, hemodynamics, and endothelium permeability. Various signaling pathways are involved, including JNK, p38, ERK and NF-κB, PKC/pleckstrin (337, 342), as well as interactions with the Toll-like–receptor pathway, as assessed by the inhibitory effect of antibodies directed against TLR4, RAGE, and CD36 (136). In macrophages and in osteoclasts, the proinflammatory effect of

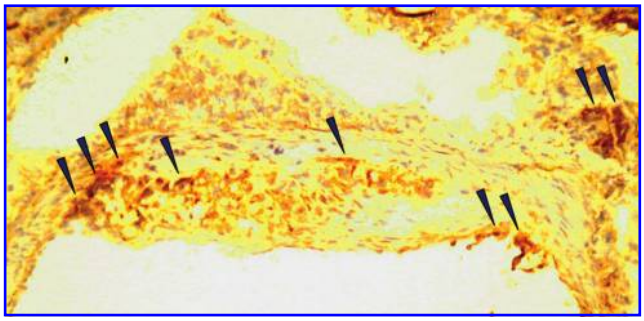

FIG. 11. Detection of CEL adducts in aortas from diabetic mice. Immunohistochemistry experiments showing the presence of CEL-adducts in aortic atherosclerotic plaques of apoE$^{-/-}$ mice rendered diabetic by streptozotocin treatment (44). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

HMGB-1 involves the upregulation of receptor activator for NF-κB ligand (RANKL) mRNA *via* interactions with TLR2 and TLR4 (364). Likewise, AGEs accumulation enhances osteoclastogenesis, *via* the upregulation of RAGE and RANKL (106).

(h) Differentiation. The formation of tubulointerstitial lesions in diabetic nephropathy could be due in part to the RAGE- and TGF-β–dependent transdifferentiation of epithelial cells into myofibroblasts, a mechanism blocked by anti-RAGE and anti–TGF-β antibodies (74).

Conversely, the upregulation of RAGE in preosteoblastic MC3T3-E1 cells impairs their differentiation in osteoblasts, which suggests a role for RAGE in the mechanism of osteoporosis in diabetes (240).

(i) Thrombosis. AGEs exert prothrombotic properties characterized by an enhanced expression of tissue factor (147, 342). Moreover AGEs trigger a decrease in prostacyclin (PGI_2), an antithrombogenic prostanoid and an increase in PAI-1 production, these effects being reversed by antisense DNA directed against RAGE mRNA (350).

(j) Apoptosis. Several AGEs are cytotoxic to cultured cells, particularly glyceraldehyde-derived AGEs (AGE-2 or TAGEs), which play a major role in the pathogenesis of angiopathy in diabetes patients and in neurodegeneration (288). AGEs induce apoptosis in various cell types, such as HUVECs (210) trophoblasts, peritoneal mesothelial cells (26), pericytes (349), osteoblasts (208), and neurons (236). Transgenic diabetic mice expressing a cytoplasmic domain-deleted RAGE in endothelial cells or mononuclear phagocytes exhibit a net reduction of ischemic injury and apoptosis markers (36). The apoptotic signaling involves oxidative stress, STAT5, and FOXO4 transcription factors (9, 62), Smad/TGF-β (183), JNK, p38, and caspase-3 activation (10, 342).

(k) RAGE-mediated immune responses. RAGE are thought to activate the innate and adaptive immune systems, because (a) RAGE are expressed on cells of the immune system, T lymphocytes, monocytes, and macrophages (357); and (b) binding of AGEs to RAGE triggers NF-κB activation and subsequent expression of inflammatory cytokines and adhesion molecules (185). The role of RAGE in the innate immune response is supported by the fact that its upregulation in macrophages leads to increased endocytic and phagocytic activity (187) and promotes the induction of a proinflammatory macrophagic phenotype (although RAGE do not interact with ligands from pathogens) (166). Moreover, $RAGE^{-/-}$ mice are protected against the lethal septic shock (185).

The role of RAGE in the adaptive immune system is debated. Liliensek and colleagues (185) reported a lack of involvement because sRAGE exhibits similar protective effects on experimental autoimmune encephalomyelitis in $RAGE^{-/-}$ and wt mice models. In contrast, Chen *et al*. (59) reported that RAGE are involved in the adaptive immune system, on the basis of experiments showing that (a) islet allograft rejection is reduced in $RAGE^{-/-}$ mice, (b) recurrent autoimmune diabetes in NOD mice is inhibitable by the RAGE antagonist TTP488, and (c) RAGE are involved in the differentiation of T cells along a Th1 phenotype (59).

b. RAGE-independent effects

(1) Biologic consequences of AGEs formation on extracellular matrix. Extracellular matrix (ECM) is formed of proteins with slow turnover rate, such as collagen, elastin, glycoproteins, and proteoglycans, which maintain the integrity and the elasticity of the vessel wall. Metalloproteases (collagenases, elastases, gelatinases) produced by vascular cells and macrophages, degrade ECM through their collagenolytic and elastolytic activities (367).

The formation of cross-links between glucose or AGEs precursors and ECM proteins alters the turnover of ECM proteins, decreases the flexibility and the permeability of large arteries, and results in a general dysfunction of collagenous tissues in diabetes patients (14, 268). Glycated collagen is associated with endothelial cell senescence and apoptosis (342).

The binding of heparin sulfate proteoglycan (HSPG) to ECM components (120, 276) decreases the ECM/proteoglycans interactions, which disrupts matrix integrity, cell/ECM interactions, cell adhesion, and migration (276). AGEs formation on arginine residues of type I collagen inhibits the adhesion and the spreading of cells (254), whereas laminin and collagen IV glycation impairs the adhesion to endothelial cells. Likewise, the adhesion and the proliferation of pericytes are impaired on glycated ECM produced by endothelial cells in high glucose concentrations (21). In addition, collagen glycation increases the adhesiveness of neutrophils and inhibits their chemotaxis and chemokinesis, thereby contributing to weakening their host-defense capacity in diabetes patients (328). The modification of arginine on collagen inhibits the binding of the $\alpha 2\beta 1$ integrin (the sole collagen-binding integrin in platelets), thus impairs platelet deposition on collagen in damaged vessels (14). Last, collagen glycation results in premature endothelial senescence and apoptosis, possibly involved in diabetic vasculopathy; these mechanisms are related to an inefficiency of autophagy, resulting (among others) from lysosomal permeabilization and nonfusion with phagosomes (253).

(2) Biologic consequences of AGEs formation on cellular proteins. AGEs can modify tissue and cellular proteins and alter their function independent of any AGE/RAGE interactions and oxidative stress. The AGEs precursors MGO and GO induce *in vitro* the formation of cross-links on tyrosine kinase receptors, such as epidermal growth factor receptor (EGFR) (262) and platelet-derived growth factor-β (PDGFR-β) (44), which impairs their activation by their own ligand and the subsequent cell migration, survival, and proliferation. MGO blocks cell proliferation *via* the activation of checkpoint kinases Chk1 and Chk2, which results in G_2/M arrest (157) and downregulates the expression of the protein-serine/threonine kinase Raf1, involved in growth and development signaling (83).

Besides the functional abnormalities resulting from insulin glycation, MGO contributes to the pathogenesis of insulin resistance by altering insulin-induced signaling, such as the insulin-stimulated phosphorylation of protein kinase B and ERK1/2 (277). This mechanism could impair insulin-stimulated glucose uptake and insulin signaling in adipose tissue of fructose-fed rats, which exhibit increased endogenous MGO accumulation correlated with insulin resistance (152). In addition, AGEs precursors strongly inhibit the activity of cellular phosphotyrosine phosphatase (262).

MGO forms glycated adducts (5-hydro-5-methylimidazolone and argpyrimidine) on eNOS, but recent studies indicate that these adducts are not inhibitory for eNOS activity (31). On the contrary, MGO could activate eNOS and the generation of NO (55, 272).

MGO and GO trigger the generation of ROS, RNS, and cross-linked protein radicals (120, 155). MGO alters the cellular antioxidant defenses by depleting the glutathione content and by inhibiting GPX-1 and glyoxalase activities, which aggravates the proapoptotic stress (79). Mechanistically, the interactions of MGO with cellular proteins stimulate NADPH oxidase (24) and the mitochondrial respiratory chain, resulting from an alteration (by MGO) of the complex III function, correlated with the generation of hydroimidazolone adducts on mitochondrial proteins (280). MGO is cytotoxic for several cell types, peripheral nerve–derived Schwann cells (112), osteoblasts (53), liver and colon cells (291), and mesangial and endothelial cells (52, 140). The cytotoxic and proapoptotic signaling of MGO involves JNK and p38 MAP kinase (53). Interestingly, MGO suppresses the activation of NF-κB mediated by TNF-α, which results in apoptosis (173).

(3) Biologic consequences of AGEs formation on DNA. AGEs-induced DNA modification and genotoxicity have been documented in bacteria, in mammalian cells, and in patients with renal failure (292, 311). AGEs-modified nucleotides in mammalian cells include GO-modified deoxyguanosine (GO-dG) and 8-hydroxydeoxyguanosine (323).

AGEs-modified nucleotide formation in DNA is associated with reduced DNA synthesis, mutations, DNA-strand breaks, and cytotoxicity (98, 323, 324). H_2O_2 enhances the mutagenic effect of MGO (98). It may be noted that only very high concentrations (>100 m*M*) of monosaccharides induce nucleotide modification and DNA strand breaks (190). However, increased levels of 8-hydroxydeoxyguanosine are observed in lymphocytes from diabetes patients (190), this being potentially associated with depletion of the cellular GSH content and DNA oxidation (303).

(4) Biologic consequences of AGEs-modified lipids. Aminophospholipid modifications by AGEs alter the biosynthesis and turnover of membrane phospholipids and disturb the physical properties of membranes, with consequences such as (a) asymmetric distribution of aminophospholipids in membranes, (b) translocation between and lateral diffusion in the membrane, and (c) activity of membrane-bound proteins that require aminophospholipids for their function (104, 250).

D. Glycation and AGEs in the pathophysiology of diabetes complications

1. Pathophysiologic role of dietary AGEs. AGEs uptake from food contributes to AGEs accumulation [reviewed by Xanthis and colleagues (345)]. Dietary AGEs (like endogenous AGEs) exhibit prooxidant and proinflammatory properties that play a role in diabetes and its complications. Exogenous AGEs (or glycotoxins) are formed during thermal food processing, at very high temperature (when food is microwaved, fried, broiled, or when it is processed for industrial treatments such as sterilization and lyophilization). The formation of dietary AGEs (evidenced by the typical "browning" responsible of the color and flavor of cooked foods) is increased in the presence of trace metals, smoke, and large amounts of carbohydrates, lipids, and proteins (56). The highest dietary AGEs content is observed in fat-rich foods (cheese, creams, butter), in animal products and in processed bread, snacks, and cereals, whereas the lowest AGEs content is observed in fresh fruits and vegetables and unprocessed food (345).

Only 10% of dietary AGEs are absorbed by intestinal cells, and among them, 30% are excreted by the kidneys whereas 70% are stored in tissues (169). Increased levels (>65%) of plasma and urinary AGEs are found in healthy rats and diabetic (nonobese or db/db), fed with a high-AGEs diet (138, 294). Conversely, studies in healthy human subjects show a correlation between AGEs dietary intake and the level of circulating AGEs (332). Likewise, nutritional intervention studies in diabetes subjects indicate that the levels of circulating AGEs are partly dependent on dietary AGEs, high AGEs intake being associated with more elevated inflammatory and proatherogenic responses (assessed by increased levels of TNF-α and C-reactive protein) (42, 332, 335). In contrast, a low-AGEs diet decreases the levels of plasma AGEs and of C-reactive protein in diabetes patients (332). In diabetic mice, an AGEs-rich diet is associated with an increased incidence of nephropathy and atherosclerosis (40). Furthermore, a daily AGEs-rich diet increases the glycation of circulating LDLs in diabetes patients and render them highly cytotoxic for cultured endothelial cells. In contrast, a low-AGEs diet ameliorates the AGEs content and the toxicity of LDL (42) and protects against arterial stent restenosis in diabetes patients (345). In mice, a reduced AGEs diet preserves the antioxidant defenses, decreases the tissue damage, and increases the lifespan (40).

Dietary AGEs can be reduced by the preferential intake of foods with low AGEs content, by more healthful cooking methods (which minimize the production of AGEs), and by high antioxidant intake, which could prevent AGEs formation (345). It has been suggested that carnivorous diets containing L-carnosine and related peptides such as acetyl-carnosine, homocarnosine, and anserine, could ameliorate glycation and diabetes complications (134). L-Carnosine is an efficient carbonyl scavenger and antiglycating agent, which inhibits the formation of cross-links induced by sugars and AGEs precursors. However, no strong evidence supports a beneficial role for dietary carnosine supplementation in diabetes complications (134).

2. Accelerated diabetic atherosclerosis. Evidence supports the implication of glycation, AGEs accumulation, and AGE/RAGE interactions in accelerated atherosclerosis and in cardiovascular diseases progression, which represent the major cause of mortality in diabetes patients.

High levels of glycated and AGEs-modified LDLs in diabetes patients (318) are associated with increased endothelium activation, inflammation, permeability, and transcytosis, whereas they promote local oxidative stress and LDL oxidation (50, 306) (Fig. 10). Glycated and AGEs-modified LDLs are taken up by the scavenger-receptor pathway (360), which promotes the formation of foam cells, and atheromatous plaques (306).

The oxidative stress generated by AGE/RAGE interactions participates (among others) to increase LDL oxidation. Moreover, oxidative stress contributes to decrease NO bioavailability and to increase $ONOO^-$ generation implicated in

endothelium dysfunction (306). The increased expression of RAGE in macrophages of vulnerable atherosclerotic plaques from diabetes patients, and their colocalization with the inflammatory markers COX-2 and MMPs, support their potential implication in plaque destabilization and rupture (63). Last, the glycation of HDLs, which strongly alters their atheroprotective properties, contributes to increasing the accelerated atherosclerosis phenomenon.

Animal models have been used for testing different therapeutic approaches allowing the limitation of the development of the lesions, such as the AGEs cross-link breaker ALT-711, inhibitors of AGEs formation such as aminoguanidine (101), PPAR-γ activators such as rosiglitazone (180), or the angiotensin-converting enzyme–inhibitor perindopril, which reduces the atherosclerosis extent, the vascular expression of angiotensin-converting enzyme, and VCAM-1 (43) (Fig. 11). The administration of sRAGE to STZ-treated apoE-null mice reduces the level of inflammation markers (COX-2, MMPs, VCAM-1, MCP-1, and tissue factor), macrophage infiltration, and SMC activation in atherosclerotic lesions (37, 163).

3. Arterial stiffening. Glycation and AGEs-induced modification of ECM proteins, in particular collagens, decrease their turnover and result in arterial stiffness and intima–media thickness (25). AGE/RAGE interactions stimulate the biosynthetic activity of endothelial cells, by increasing the expression of fibronectin and collagen IV and ECM accumulation, resulting in basement membrane thickening and fibrosis (69, 306). AGE/RAGE interaction stimulates the neointimal expansion by inducing activation and proliferation of SMCs (365, 367). Vascular stiffening alters the elasticity of large arteries and induces increased systolic pressures, with deleterious consequences on the heart, including cardiac hypertrophy and increased ventricular oxygen consumption (174).

4. Restenosis. Angioplasty is used largely to treat arterial stenosis affecting coronary, carotid, renal, and peripheral (mainly leg) arteries. This procedure induces a vascular response to injury, which results in SMC migration, proliferation, and the secretion of ECM proteins. The incidence of postangioplasty restenosis, and other complications, is much higher in diabetes patients (73, 225). Neointimal hyperplasia induced by carotid balloon injury in obese diabetic Zucker rats is inhibited by sRAGE, which reduces SMC proliferation (251). Acute endothelial injury in femoral artery of wild-type mice induces neointimal hyperplasia associated with upregulation of RAGE in SMCs of the injured vessel, which is reduced in animals treated with sRAGE (281). Homozygous RAGE-knockout mice and transgenic mice expressing dominant negative RAGE selectively in SMCs and subjected to arterial denudation experiments, exhibit reduced intimal hyperplasia (225, 281, 342). All these data highlight the impact of RAGE signaling on SMC proliferation and neointima formation after injury.

5. Microvascular complications. Microvessels are composed of pericytes and of endothelial cells. Pericytes regulate endothelial cell growth and their antithrombogenic function. AGEs and RAGE are involved in the setting of microvascular lesions in diabetes, either through a direct effect on endothelial cells, or through mechanisms leading to pericyte apoptosis, and to the modification of matrix components (342). Aminoguanidine, an inhibitor of AGEs formation, and sRAGE prevent the microvascular complications in animal models, thereby assessing the implication of AGEs and RAGE in these lesions (341).

a. Retinopathy. Retinopathy is a severe vascular complication in diabetes, which may progressively lead to blindness. Apoptosis of pericytes ("pericyte loss"), is a key factor in retinopathy by triggering endothelial activation and dysfunction, which results in neoangiogenesis and thrombogenesis (342). BSA modified by various AGEs precursors (MGO, GO, glyceraldehyde, 3-deoxyglucosone) may induce growth arrest and apoptosis of pericytes (77), whereas it is mitogenic for endothelial cells and promotes neoangiogenesis (cell proliferation and tube formation), *via* an upregulation of VEGF (342). AGEs stimulate the secretion of the prothrombotic PAI-1, whereas they inhibit the production of the antithrombotic prostacyclin PGI_2, thereby promoting thrombogenesis (348). These events are potentiated by increased RAGE expression, as shown in retinal Müller glia, which regulate the vascular permeability and the cellular response to stress (340).

b. Nephropathy. Diabetic nephropathy is characterized by the presence of a persistent proteinuria (>0.5 g/24 h), and by a progressive decline in renal function, resulting in end-stage renal disease. AGEs play a key role in glomerular nephropathy as they accumulate in glomerular basement membrane and interact with mesangial cells, endothelial cells, and podocytes, to trigger oxidative stress, inflammatory signaling, and apoptosis (110, 282). Wautier and Schmidt (342) stressed the fact that podocytes express high levels of RAGE (contrary to mesangial and endothelial cells) and podocyte injury and apoptosis, leading to proteinuria and glomerular injury (219).

Oxidative stress and the secretion of growth factors and cytokines are involved in AGEs-induced nephropathy and are entangled with the activation of the renin–angiotensin system, which also generates ROS and growth factors [for review, see (110) and Forbes *et al*. (100)]. Animal-model experiments support the role of oxidative stress and RAGE in diabetic nephropathy. First, increased glomerular permeability and diabetic nephropathy are aggravated by oxidative stress, which induces modifications of endothelial cell shape *via* calcium-mediated mechanisms (342), and are inhibited by antioxidants (320) and by inhibitors of AGEs formation, such as aminoguanidine (212, 342). Second, RAGE blockade ameliorates albuminuria and mesangial expansion in diabetic db/db mice and in STZ-treated wild-type mice. Third, diabetic vascular diseases are suppressed in homozygous RAGE-null mice (342). Transgenic mice overexpressing human RAGE in vascular cells exhibit albuminuria, increased serum creatinine and glomerulosclerosis, and a general predisposition to nephropathy (353). Finally, a recent report shows the interaction between the renin–angiotensin system and AGE/RAGE, because benazepril, an angiotensin-converting enzyme inhibitor (ACEI), suppresses AGEs accumulation, RAGE expression, NF-κB activation, inflammatory signaling, and oxidative stress, and slows the nephropathy of spontaneously hypertensive rats (188). However, despite positive preclinical research findings, several tested antioxidants have shown low renoprotection in humans (100).

c. Neuropathy. Peripheral neuropathy is a common diabetes complication associating nerve dysfunction and loss of pain perception, associated with an increased risk for developing ulcerations and necrosis, particularly diabetic foot, and impaired wound healing (potentially leading to lower-limb amputation) (144). RAGE and AGEs are implicated in the loss of pain perception, *via* several mechanisms. Oxidative stress and NF-κB sustained activation induce the expression of proinflammatory genes, resulting in neurologic dysfunction and altered pain sensation, as shown by diabetic RAGE-deficient mice that do not undergo loss of pain, in contrast to wild-type mice (144, 312). The loss of pain could result from the modification by AGEs of axonal cytoskeletal tubulin, neurofilament, ECM protein laminin, and actin. This leads to axonal degeneration, impaired axonal transport, and segmental demyelination of peripheral nerves, which become prone to phagocytosis by macrophages (312). In diabetes patients, AGEs (CML) and RAGE colocalize with NF-κB and IL-6 in mononuclear $CD4^+$, $CD8^+$, and $CD68^+$ cells, that invade the nerves in epineurial and endoneurial vessels and the perineurium (127). Studies on experimental diabetic neuropathy show that RAGE-expressing animals exposed to long-term diabetes exhibited neuropathy correlated with a dramatic increase in RAGE expression in peripheral epidermal and sural axons, Schwann cells, and sensory neurons, whereas $RAGE^{-/-}$ mice exhibited attenuated neuropathy (327). Recombinant sRAGE and aminoguanidine could prevent microvascular vasculopathies and nerve lesions (312, 341). However, therapeutic strategies with anti-AGEs agents still must be established for preventing neuropathies and their consequences (312).

d. Wound healing. Impaired wound healing is a frequent diabetes complication, as a result of diabetic neuropathy, vasculopathy, decreased O_2 delivery to tissues because of hemoglobin glycation, and alteration of innate immune mechanisms (121). AGEs and RAGE largely participate in wound-healing impairment through oxidative stress, decreased chemotaxis of phagocytes and decreased phagocytosis into the wound, increased secretion of proinflammatory cytokines (IL-1, IL-6, TNF-α), increased secretion of MMPs, and reduced synthesis of collagen by fibroblasts (121, 122). The glycation of FGF_2 inhibits its capacity to induce endothelial cell proliferation, tube formation, and angiogenesis promotion (88). Fibroblast apoptosis plays a role in altered wound healing, which, when combined with osteoblast apoptosis, contributes to the enhanced risk of periodontal and bone diseases in diabetes (122). In diabetes patients, prolonged accumulation of AGEs within the skin and subcutaneous elements may delay the entry of inflammatory cells into the wound, whereas RAGE-bearing phagocytes and effector cells react with AGEs into the wound, leading to reinforcement of the local cellular activation and inflammation, and finally, to wound-healing impairment (121). Dietary AGEs may also alter wound healing, as reported for diabetic mice fed on a high-AGEs diet (which exhibit delayed wound healing) (255). Administration of sRAGE efficiently reduces the levels of AGEs, RAGE, macrophages, proinflammatory molecules, and MMPs in wounds (121).

6. Alzheimer's disease. Alzheimer's disease (AD) is a common cause of dementia in developed countries. It is characterized by the presence of senile plaques and neurofibrillary tangles, resulting from the accumulation of amyloid-β and tau protein. Diabetes may aggravate the pathogenesis of Alzheimer's disease, through the glycation of tau, which is implicated in the formation of the paired helical filaments (a component of the neurofibrillary tangles) and the glycation of amyloid-β protein, which markedly enhances its aggregation. Amyloid-β peptide and β-sheet fibrils are additional ligands for RAGE, which triggers oxidative stress, inflammation, and amyloidosis and amplifies the neurotoxic effect of amyloid-β peptide on microglia, the blood–brain barrier, and neurons (58).

A number of AGEs-related crosslinks, such as pyrraline, pentosidine, CML, GOLD and MOLD, and the neurotoxic TAGEs, are detected in senile plaques (317). TAGEs are found in the cytosol of neurons and interfere with axonal transport and intracellular protein traffic (317). TAGEs are implicated in the neurotoxic effect of serum from diabetes patients for cultured neurons, which is reversed by anti-TAGEs antibodies (349). The level of TAGEs in the cerebrospinal fluid (349) or of pentosidine in the serum (207) represents promising biomarkers for the early detection of AD.

7. Cancers. RAGE and RAGE ligands, such as S100/calgranulins and HMGB1, are expressed and secreted by cancer cells in a wide variety of tumors [for review, see (189)]. Their presence indicates a poor prognosis, as they can stimulate proliferation, metastasis, and chemoresistance of cancer cells, but also of the other cell types present in the tumoral microenvironment (fibroblasts, leukocytes, vascular cells), which can aggravate inflammation and angiogenesis. Moreover, cells present in the tumor environment produce RAGE ligands, which are associated with increased risks of metastasis. *In vitro* studies reported that AGE/RAGE interactions stimulate the growth of human pancreatic cancer cells and of cultured human melanoma cells, whereas anti-RAGE antibodies inhibited tumor formation and lung metastasis of melanoma cell xenografts and improved the survival of athymic mice. In humans, elevated circulating AGEs and DNA glycation levels represent an increased risk factor for colorectal cancer in diabetes patients (323, 349).

Drug-resistant tumor cells (human leukemia, lung carcinoma cells) overexpress glyoxalase I, which confers resistance to drug-induced apoptosis. Glyoxalase I overexpression also is observed in ovarian and breast cancer (323) Note that the permeable glyoxalase I inhibitor *S-p*-bromobenzylglutathione cyclopentyl diester countered drug resistance conferred by glyoxalase I overexpression and exhibited potent antitumoral properties against lung and prostate carcinoma (323).

8. Osteoporosis. Osteoporosis results from the reduction of bone mineral density, which triggers bone fragility and fractures. Diabetes patients have an increased risk of developing osteoporosis. Among the mechanisms linking osteoporosis and diabetes, AGEs and AGE/RAGE interactions exert a deleterious effect on osteoblast function. The upregulation of RAGE in preosteoblastic MC3T3-E1 cells impairs their differentiation in osteoblasts (240). AGE/RAGE interactions decrease osteoblast proliferation, alkaline phosphatase activity, and type 1 collagen production, as well as the expression of transcription factors implicated in osteogenesis (349). AGEs form crosslinks on bone matrix proteins, which alters their ability to induce bone formation and to interact

with bone-inductive proteins such as bone morphogenetic protein-2. Moreover, AGEs trigger osteoblast apoptosis and stimulate bone resorption by osteoclasts in cultured mouse unfractionated bone cells (349) *via* the upregulation of RAGE and RANKL (106). In MC3T3E1 mouse calvaria–derived osteoblasts, AGEs alter the expression and secretion of galectin-3, a multifunctional protein with antiapoptotic and carbonyl-scavenger properties, thereby contributing to osteoblast apoptosis (209). Moreover, AGE/RAGE interactions induce human mesenchymal stem cell apoptosis, thereby inhibiting their differentiation into adipose tissue, cartilage, and bone (349). Decreased bone formation, associated with the other diabetes complications and aging, increase the risk of fractures in diabetes patients.

Low doses of bisphosphonates (alendronate, pamidronate, or zoledronate), usually used for the treatment of osteoporosis, reverse the deleterious effect of AGEs on osteoblasts (115). Metformin, an antiglycating agent, and pyridoxamine (a post-Amadori inhibitor) exhibit osteogenic properties and stimulate osteoblast proliferation and mineralization *in vitro*, but their efficiency *in vivo* is debated (70, 349).

IV. Conclusions and Perspectives

During the past two decades, considerable advances have emerged in the understanding of hyperglycemia-induced cell and tissue damage and their consequences in diabetes complications. These studies allowed highlighting more precisely the role of (a) the glycation process and AGEs formation/accumulation on proteins and tissues, and (b) oxidative stress, which represents a common link shared by the different "high glucose"–associated situations. If the pathophysiologic basis of acute stress hyperglycemia is still unclear, the mechanisms linking chronic hyperglycemia and oxidative stress to glycation and AGEs formation and signaling have been particularly studied. Oxidative stress is, *via* hyperglycemia and AGEs, a determinant and threatening factor in the pathobiology of diabetes complications (35, 320, 350). Conversely, AGEs formation is an inexorable reaction in all living systems that leads to structural and functional changes on proteins (19, 326), such as (a) alterations in their physicochemical properties (conformation, charge, hydrophobicity, elasticity, solubility, electrophoretic mobility); (b) formation of intra- and intermolecular protein crosslinks and aggregates; (c) decrease/inhibition in enzyme activity; (d) alterations of peptide hormone signaling; (e) alteration of protein degradation (*i.e.*, resistance to proteolysis); (f) altered trafficking and processing of proteins; (g) modification of ECM and cell-matrix interactions; (h) activation of AGEs-specific receptors; and (i) stimulation of autoimmune responses.

However, whether some key cellular or extracellular proteins are preferential targets and whether the extent of their modification is sufficient for explaining impaired cellular and tissue function, remains an open question. In this context, it is still difficult to discern which target proteins are specifically damaged and the existence of an AGEs-proteome of diabetes. Many specific proteins—from blood and tissues containing long-lived proteins, such as ECM, cartilage, dura mater, and lens—have been described (see Tables 2, 3, and 4). Obviously, more studies are needed for evaluating the structural/functional factors involved in their specific AGEs modification, their consequences, and their role in the pathobiology of diabetes complications. Reactive carbonyl species probably act in a random fashion.

Besides the classic medical treatment of diabetes, a number of therapeutic approaches for neutralizing AGEs have been investigated. So far, studies using AGEs inhibitors (aminoguanidine or pyridoxamine), gave interesting but debated results in animals and were not conclusive in humans (259). Some benefits in humans have been obtained with the AGEs breaker 4,5-dimethyl-3-phenacylthiozolium chloride (ALT-711), which improves arterial compliance and decreases pulse pressure [see review by Wautier et al. (342)]. Clinical studies with statins, angiotensin II, or ACE inhibitors are under investigation, and should clarify whether their use is of benefit in diabetes complications such as retinopathy and accelerated atherosclerosis (350). ApoA1-mimetic peptides, such as L-4F, have been recently developed and successfully tested in animal models for atherosclerosis and diabetes. These peptides exert an antioxidant and antiapoptotic activity by increasing heme-oxygenase-1, pAMPK, and e-NOS activities, thereby ameliorating the vascular function in diabetic animals (257, 258). Last, sRAGE, by inhibiting the binding of AGEs to RAGE, suppresses accelerated atherosclerosis and nephropathic complications in mice, and may represent a suitable therapeutic target for the prevention of diabetic vascular complications in the future (342, 350). In the next few years, the clinical efficacy of these promising therapies will be evaluated in the clinical context and should improve the prognosis of diabetic complications (118).

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Abbreviations Used

AGEs = advanced glycated end products
AP-1 = activator protein-1
CCR2 = CC chemokine receptor 2
CEL = *N*ε-(carboxyethyl)lysine
CETP = cholesteryl ester transfer protein
CML = *N*ε-(carboxymethyl)lysine
COX2 = cyclooxygenase 2
DAG = diacylglycerol
3-DG = 3-deoxyglucosone
ERK = extracellular signal-regulated kinase
FFA = free fatty acid
GAPDH = glyceraldehyde-3 phosphate dehydrogenase
GFAT = L-glutamine:D-fructose-6-phosphate amidotransferase
GO = glyoxal
GPx = glutathione peroxidase
GSH = glutathione (reduced form)
GSSG = glutathione, oxidized form
GST = glutathione *S* transferase
HDL = high-density lipoprotein
H_2O_2 = hydrogen peroxide
ICAM-1 = intercellular adhesion molecule-I
IκB = NF-κB inhibitor
IL = interleukin
JNK = c-Jun N-terminal kinase
LCAT = lecithin-cholesterol-acyl transferase
LDL = low-density lipoprotein
LRP1 = low-density lipoprotein-related protein 1 (α_2-macroglobulin receptor)
MCP-1 = monocyte chemotactic protein-1
MGO = methylglyoxal
MHC = major histocompatibility complex
Mn-SOD = manganese-dependent superoxide dismutase
NF-κB = nuclear factor-κB
NO = nitric oxide
$ONOO^-$ = peroxynitrite
PAI-1 = plasminogen activator inhibitor-1
PARP = poly(ADP-ribose) polymerase
PBMC = peripheral blood mononuclear cell
PGI_2 = prostacyclin
PKC = protein kinase C
PPAR-γ = peroxisome proliferator–activated receptor-γ
RAGE = receptors for advanced glycated end products
ROS = reactive oxygen species
SMC = smooth muscle cell
SOD = superoxide dismutase
SP1 = specificity protein 1 transcription factor
TAGEs = toxic advanced glycation end products
TCA cycle = tricarboxylic acid cycle
TGF-β = transforming growth factor-β
TLR = Toll-like receptor
TNF-α = tumor necrosis factor-α
tPA = tissue-type plasminogen activator
UCP-1 = uncoupling protein 1
VCAM-1 = vascular cell adhesion molecule-1
VEGF = vascular endothelial growth factor
VLDL = very low density lipoprotein

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