The process of nonenzymatic glycation and oxidation of proteins yields a diverse array of modified products. AGEs are a heterogeneous group of compounds formed via endogenous and exogenous mechanisms. Increased levels of glucose drive the formation of Schiff bases; Schiff bases subsequently rearrange to form the more stable Amadori products. These reactions are reversible, and the levels of Amadori products are directly related to the levels of glucose. Once Amadori products form, further oxidative modifications of these molecules may occur, resulting in the formation of the irreversible advanced glycation end products (AGEs). In settings in which oxidative processes are involved in the formation of these AGEs, so-called glyoxidative species such as \( \alpha \)-carboxymethyllysine (CML) and pentosidine form.1,2

Glycolytic intermediates are intracellular sources of AGEs. In these more rapid Maillard-type reactions, 3 classes of dicarbonyl compounds may form, which are potent glycation agents, including glyoxal, methylglyoxal (MG), and 3-deoxyglucosone. Among these, the most potent glycation agent is likely MG. MG forms via the conversion of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate and principally reacts with arginine groups to form AGEs such as 5-hydro-5-methylimidazolone and the fluorescent AGE, argpyrimidine.1 MG levels can be regulated via the activity of 2 enzymes, glyoxalase I and II; these enzymes detoxify MG and result in MG conversion to d-lactate.4 When the level of the activity of glyoxalases is impaired, MG levels rise, thereby favoring further AGE production.5

In addition to glucose or glycolytic intermediate–derived AGEs, lipid peroxidation may result in the formation of reactive carbonyl compounds capable of interacting with proteins, thereby leading to the production of advanced lipoxidation end products. Of note, CML is one of the advanced lipoxidation end products; CML-modified adducts may form both by glycoxidation and by lipid peroxidation pathways. Furthermore, when MG reacts with lysine residues of proteins, a homolog of CML-AGE may be produced, known as \( \alpha \)-carboxyethyllysine.6 CML-AGE may also be formed by inflammatory mechanisms driven by the myeloperoxidase pathway.7 Also, natural aging and renal failure,4,8–10 in the absence of other risk factors such as diabetes mellitus, are associated with endogenous AGE formation and accumulation.

Exogenous sources of AGEs may contribute to AGE formation in vivo. Foods high in fat and those cooked at high temperatures contain AGEs, the most prominent of which is CML-AGE and the AGE precursor, MG.11,12 Furthermore, air pollution–associated fly ash was shown to lead to the generation of AGEs on proteins in fibroblasts exposed to this agent for long periods of time.13 Hence, dietary and environmental

**Key Words:** advanced glycation end products ■ receptors ■ insulin resistance ■ type 2 diabetes mellitus

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**Abstract**—Multiple biochemical, metabolic, and signal transduction pathways contribute to insulin resistance. In this review, we present evidence that the posttranslational process of protein glycation may play a role in insulin resistance. The posttranslational modifications, the advanced glycation end products (AGEs), are formed and accumulated by endogenous and exogenous mechanisms. AGEs may contribute to insulin resistance by a variety of mechanisms, including generation of tumor necrosis factor-\( \alpha \) direct modification of the insulin molecule, thereby leading to its impaired action, generation of oxidative stress, and impairment of mitochondrial function, as examples. AGEs may stimulate signal transduction via engagement of cellular receptors, such as receptor for AGEs. AGE–receptor for AGE interaction perpetuates AGE formation and cellular stress via induction of inflammation, oxidative stress, and reduction in the expression and activity of the enzyme glyoxalase I that detoxifies the AGE precursor, methylglyoxal. Once set in motion, glycation-promoting mechanisms may stimulate ongoing AGE production and target tissue stresses that reduce insulin responsiveness. Strategies to limit AGE accumulation and action may contribute to the prevention of insulin resistance and its consequences. (Arterioscler Thromb Vasc Biol. 2012;32:1760-1765.)
sources of AGEs may represent additional key depots for the formation of these products. When detoxification mechanisms (such as glyoxalases) or clearance mechanisms, such as in advanced renal disease, are suppressed, AGE levels and their consequences may be amplified.

**Glycation Products, Receptors, and Mechanisms to Sustain and Enhance AGE Levels**

One of the main mechanisms by which AGEs exert their pathogenic effects is via interaction with cell surface receptors. There are multiple receptors for AGEs such as receptor for AGE (RAGE), CD36, lectin-like oxidized low-density lipoprotein receptor-1, macrophage scavenger receptors, or receptor for advanced glycation endproducts. RAGE is a signal transduction receptor for AGEs. These receptors may contribute to AGE formation and accumulation. For example, when AGEs bind to RAGE, signal transduction mechanisms are instigated in a process requiring the cytoplasmic domain of the receptor. A chief outcome of AGE-RAGE signaling is the generation of oxidative stress, largely through the nicotinamide adenine dinucleotide phosphate-oxidase system. Because oxidative stress facilitates AGE formation, one consequence of the interaction of AGE with RAGE is the generation of an environment that favors further AGE production. Thus, it was not surprising that in mice devoid of RAGE, levels of oxidative stress and AGEs were lower compared with wild-type RAGE-expressing animals. It has been shown that in the kidney of diabetic OVE26 mice devoid of RAGE, levels of glyoxalase I mRNA and protein were higher compared with those of RAGE-expressing OVE26 mice. In parallel, levels of MG and AGEs were lower in the RAGE-deficient mice, despite equal degrees of hyperglycemia and glycosylated hemoglobin levels.

The potential roles of RAGE in AGE generation have a further layer of complexity, because RAGE is not solely a receptor for AGEs but a multiligand member of the immunoglobulin superfamily. Thus, beyond AGEs, RAGE also transduces the signals emitted by multiple members of the proinflammatory S100/calgranulin family and high-mobility group box 1. These molecules, at least in part via RAGE, upregulate cytokines, matrix metalloproteinases, and gene expression programs in monocytes/macrophages and lymphocytes that amplify tissue-damaging inflammation in settings such as autoimmunity and extensive cellular/tissue stress. Inflammation itself may generate oxidative stress; for example, one key consequence of activation of the myeloperoxidase pathway is the generation of CML-AGEs. Hence, ligand-RAGE may trigger oxidative stress and inflammation; once S100/calgranulin- and high-mobility group box 1-bearing inflammatory cells are recruited to the sites of tissue stress, release of these non-AGE ligands may exacerbate oxidative stress and stimulate further AGE formation. Figure 1 summarizes the proposed hypotheses regarding triggers to glycation and consequences of glycation, and amplifying pathways further exacerbate generation and accumulation of AGEs.

Although the earliest studies on AGEs involved their mechanistic links to the pathogenesis of diabetic complications, recent intriguing studies have suggested that glycated products and AGEs may contribute to the pathogenesis of insulin resistance as well. In the section to follow, we will present a summary of data in human subjects suggesting that AGEs may be linked to insulin resistance.

**Glycation and Links to Insulin Resistance: Studies in Human Subjects**

Several studies have reported interesting associations between AGE levels and insulin resistance, even in the absence of diabetes mellitus. Tan et al measured AGE levels, associated inflammatory markers, and insulin resistance by the homeostatic model assessment index or HOMA-IR in 207 healthy subjects without diabetes mellitus. Serum levels of AGEs correlated in a statistically significant manner with HOMA-IR in both male and female subjects. Even after adjustment for age, sex, body mass index, waist circumference, cigarette smoking, adiponectin levels, and markers of oxidative stress and inflammation, AGE levels were an independent correlate of HOMA-IR on multiple regression analysis. Tahara et al reported on 322 nondiabetic Japanese subjects; serum AGE levels correlated with HOMA-IR in these subjects, and after multiple regression analysis, AGEs, along with waist circumference, glycosylated hemoglobin, and triglycerides, were correlated with the degree of insulin resistance. When age-adjusted HOMA-IR levels stratified by AGE tertiles were compared using statistical tests, the authors found trends in both male and female subjects. Sarkar et al tested the potential association between levels of total carbonyl compounds in serum with HOMA-IR levels in type 2 diabetic subjects and found a highly significant correlation between the degree of insulin resistance and carbonyl levels, whereas levels of lipid peroxidation end products and thiobarbituric reactive substances were not significantly correlated with HOMA-IR, perhaps suggesting that pre-AGE compounds rather than the pathophysiological state of oxidative stress alone were required to impact insulin sensitivity. Diamanti-Kandarakis et al studied AGE levels in a group of subjects with polycystic ovary syndrome. Subjects with polycystic ovary syndrome often display insulin resistance. Among the 193 subjects studied, 100 had polycystic ovary syndrome, and the remaining subjects were age- and body mass index–matched control individuals. Subjects with polycystic ovary syndrome had significantly higher AGE levels than subjects with isolated hyperandrogenemia, anovulation, ultrasound diagnosed polycystic ovary, and control subjects.

Taken together, these findings in human subjects suggest potential links of AGEs to insulin resistance. Evidence suggestive of mechanistic links between AGEs and insulin resistance was provided by the results of in vitro and in vivo experimentation. In the sections to follow, we discuss these findings and suggest that AGEs might be new therapeutic targets for mitigating insulin resistance.

**Glycation and Insulin Resistance: In Vitro Evidence**

**Direct Effects of Modification of Insulin**

Recent work has suggested that glycation of insulin is a distinct possibility and that such glycation may change the properties of insulin, impacting on its action. Although insulin has a very
short half-life of 5 to 10 minutes, it has been shown that glycation of insulin and proinsulin may occur in the pancreas during the periods of insulin synthesis and storage. Boyd et al prepared monoglycated insulin to correspond to precisely what has been found in vivo, that is, single glycation modification in the phenylalanine position in the amino terminus of the insulin B chain. Compared with nonglycated control insulin, infusion of the glycated form to mice undergoing a glucose tolerance test revealed a 20% reduction in glucose-lowering potency. In isolated abdominal muscle, monoglycated insulin was 20% less effective in mediating glucose uptake, glucose oxidation, and glycogen production compared with the nonglycated form of insulin.30 In plasma pooled from 4 male human subjects with type 2 diabetes mellitus (mean glycosylated hemoglobin levels of 8.1%), Hunter et al31 showed that monoglycated insulin comprised ≈9% of the total insulin. When pure monoglycated insulin was tested in hyperinsulinemic euglycemic clamp studies, administration of glycated insulin resulted in a reduced requirement for exogenous glucose infusion to maintain euglycemia and revealed that 70% more glycated insulin was required to induce a similar amount of insulin-mediated uptake of glucose.31 Hence, in animal models and human subjects, evidence suggests that glycation of insulin may impair its action.

In distinct studies, Jia et al showed that methylglyoxal modification of insulin at an arginine residue of the insulin B chain reduced glucose uptake induced by MG-modified insulin in 3T3-L1 adipocytes and L8 skeletal muscle cells compared with native insulin. In other studies, MG modification of insulin affected insulin signaling directly. Fiory et al showed that MG modification of insulin blocked insulin receptor substrate tyrosine phosphorylation and phosphoinositide 3-kinase protein activation in the INS-1 pancreatic β-cell line. Oliveira et al showed that MG modification of insulin blocked insulin receptor substrate tyrosine phosphorylation and phosphoinositide 3-kinase protein activation in the INS-1 pancreatic β-cell line. Oliveira et al showed that MG modification of insulin blocked insulin fibril formation and led to the generation of insulin native-like aggregates. The authors speculated that this would result in diminished insulin action. It is important to note that the extent to which insulin is modified in vivo by MG modification in human subjects is not completely clarified.
Glycation of Distinct Proteins and Insulin Resistance

There are numerous published examples of protein glycation and mechanisms by which they may impact insulin sensitivity. Glycation of albumin has been shown to increase the production of tumor necrosis factor-α. Tumor necrosis factor-α has been linked to insulin resistance via induction of proinflammatory mechanisms that suppress insulin signal transduction. In L6 skeletal muscle myotubes, Cassese et al. showed that human glycated albumin induced Src-mediated activation of protein kinase C-α and suppressed IRS-1 action in a RAGE-dependent manner. Because of the diverse nature of potential targets for AGE and MG modification, Gugliucci proposed the hypothesis that MG modification of AMP kinase (AMPK) might contribute to metabolic dysfunction and hepatic insulin resistance. Gugliucci reasoned that the sensing of AMP levels by AMPK is dependent on a domain with 3 arginine residues. If any or all of those residues were modified by the potent glycating agent MG, then it is plausible that functional modification might ensue as well. Hence, if AMPK activity were reduced, the consequences might include enhanced gluconeogenesis and lipogenesis, both features that characterize hepatic insulin resistance. Although not proved experimentally at this time, the concept nevertheless raises the intriguing possibility that glycated modification of distinct biochemical moieties that contribute to regulation of insulin sensitivity beyond AMPK, such as other key metabolic enzymes, molecules involved in mitochondrial biogenesis, and molecules that modulate oxidative stress, for example, might drive further AGE- or MG-mediated microenvironments conducive to the development of insulin resistance. In this context, efforts to reduce glycation in vivo have been tested in experimental animal model systems.

Glycation and Insulin Resistance: In Vivo Evidence

Support for roles of glycation in vivo has been sought with the use of antiglycating agents to address the question, does reduction or prevention of glycation in vivo block the development of insulin resistance in vulnerable organisms? To test this concept, investigators have tested such agents in an array of animal models.

Unoki-Kubota et al. examined KK-A(y) mice, a mouse model of obesity and type 2 diabetes mellitus, and found that serum levels of AGEs correlated positively with the levels of insulin in these animals. To address whether glycation products played roles in the insulin resistance phenotype, they administered pyridoxamine, an inhibitor of AGE formation, to mice and found that the agent decreased fasting insulin levels and improved insulin sensitivity in a dose-dependent manner, but did not affect fasting blood glucose levels (at least over the short time course of administration).

Guo et al. treated Sprague-Dawley rats with MG in the drinking water to address the question of whether MG would induce insulin resistance. Two different therapeutic approaches were added to the experimental design: MG alone versus MG+N-acetyl cysteine (an antioxidant) or MG+TM2002, an inhibitor of AGEs. The animals were treated for 4 weeks and then subjected to hyperinsulinemic euglycemic clamp studies. MG administration induced insulin resistance; compared with MG alone, treatment with either N-acetyl cysteine or TM2002 completely improved the insulin resistance induced by MG. In an additional set of animals, co-treatment with MG and high-salt diet was tested. Compared with MG or high-salt diet alone, co-treatment resulted in higher systolic blood pressure, increased urinary excretion of albumin and urinary thiobarbituric reactive substances, and renal nitrotyrosine levels. Of note, the AGE derived from MG, Ne-(carboxyethyl)lysine AGE, was higher in any groups treated with MG versus groups not given MG in this study. Importantly, although these studies did not detail the precise molecular mechanisms by which MG induced insulin resistance, they nevertheless provided evidence linking MG to insulin resistance in vivo.

Kooptiwut et al. addressed these concepts directly in pancreatic islets. They retrieved islets from the pancreata of DBA/2 and C57BL/6 mice and exposed them to 11.1 or 40 mmol/L glucose concentrations alone or in the presence of the AGE inhibitor aminoguanidine. They found that chronic exposure to hyperglycemia resulted in decreased glucose-stimulated (20 mmol/L) islet secretion of insulin in the DBA/2 but not C57BL/6 islets and that this was associated with reduced glucokinase in the DBA/2 islets. When the islets were coincubated with aminoguanidine, the levels of DBA/2 glucokinase rose, thereby suggesting that chronic glucose-mediated increases in AGEs could participate in islet dysfunction over this time course, especially in DBA/2 background, and that efforts to reduce AGEs might be protective.

Finally, Matsumoto et al. reported on a model system in silkworm in which exposure of the silkworm to high glucose resulted in decreased growth, in parallel with increased AGE levels. When the silkworms were treated with high glucose and aminoguanidine, growth was restored, thereby suggesting that the accumulation of AGEs was contributing to growth suppression. Interestingly, in this model, treatment with 5-aminoimidizole-4-carboxamide riboside (stimulator of AMPK) or insulin also improved growth, thus identifying positive controls for a novel model system to test the adverse effects of high glucose and AGEs on silkworm growth.

Perspectives, Challenges, and Future Directions

Taken together, the data discussed in this review, from both in vitro and in vivo experimentation, provide supporting evidence that AGEs may contribute, at least in part, to the pathogenesis of insulin resistance by a variety of potential mechanisms, as summarized in Figure 2. The finding that AGE inhibitors, although they may have additional mechanism(s) of action beyond suppressing AGE, suppress insulin resistance in animal models identifies an entirely novel area of therapeutic possibilities for this disorder. It is important to note that in addition to endogenous formation of AGEs, high-fat diet induced AGE introduction in the diet, and environmental pollutants may be linked to AGE generation as well. Hence, dietary modification and reduction in environmental pollutants may also hold
promise for therapeutic intervention in this key area of public health concern.

It is important to note that recent studies have highlighted potentially adaptive roles for MG in the nervous system. Distler et al \(^{43}\) showed that MG is protective against anxiety; the authors showed that glyoxalase 1 increases anxiety in transgenic mice overexpressing this enzyme by reducing GABA\(_\text{A}\) receptor activation by MG. Consistent with specific roles for MG, administration of MG assuaged anxiety in the glyoxalase 1 transgenic mice. \(^{43}\)

Furthermore, data suggest that blockade of cellular receptors for AGEs might be beneficial in insulin resistance states, in which even fleeting bouts of hyperglycemia might facilitate AGE formation sufficient to trigger activation of signal transduction receptors. A clear challenge in the field will thus be to identify the specific AGEs and their levels in plasma and tissues that mediate insulin resistance versus those that trigger diabetic complications. It will be important to decipher whether differential effects of distinct types of AGEs, their levels in the tissue microenvironment, and possible receptor interactions differ in these settings.

Finally, the role of AGE–receptor interaction might be critical to address in therapeutic strategies for additional reasons. Specifically, AGE–receptor interaction has been implicated in the late stage steps in insulin resistance in which accruing damage to the pancreatic β-cell eventually overwhelms the capacity of the islet to compensate for such stress, thereby resulting in hyperglycemia and diabetes mellitus. AGEs have been shown to damage pancreatic β-cells via oxidative stress in a process that appears to require RAGE. \(^{44,45}\)

To what extent AGE precursors and AGEs are implicated in the development of insulin resistance and type 2 diabetes mellitus in vivo is an important question and one worthy of investigation. As the incidence of obesity and insulin resistance continues to rise in adolescents and adults at staggering levels, new approaches to tackling this worldwide epidemic are warranted.

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**Disclosures**

None.

**References**


Glycation and Insulin Resistance: Novel Mechanisms and Unique Targets?
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다양한 생화학적, 대사적 신호전달 체계는 인슐린 저항성 발생에 기여한다. 여러 연구 결과들은 단백질의 당화반응이 전사 후 과정에서 인슐린 저항성에 중요한 역할을 한다는 증거들을 보여주고 있다. 전사 후 변형과정을 통해 advanced glycation end products (AGEs)는 내인적 또는 외인적 기전에 의해 형성 및 축적된다. AGEs는 다양한 기전을 통한 인슐린 저항성에 기여하게 되는데, 예를 들면 TNF-α 생성을 통해 직접적으로 인슐린 분자를 변형시켜, 그 기능에 이상을 가져오고 산화 스트레스의 생성, 미토콘드리아의 기능 부진 등을 가져오게 한다. 또한 AGEs는 AGEs 수용체와 같은 세포의 수용체를 통해 신호전달체계를 자극하게 된다. AGE와 AGE 수용체의 상호작용은 염증, 산화 스트레스의 유도 및 AGE의 전구물질인 methylglyoxal을 해독하는 효소인 glyoxalase의 활성 및 발현을 감소시켜 AGE 생성 및 세포 내 스트레스를 가속화시킨다. AGE 축적 및 역할을 제한하는 치료 전략은 인슐린 저항성 및 그로 인한 여러 문제점을 예방하는데 기여할 것으로 생각된다.

단백질의 비효소적 당화반응과 산화과정은 다양한 변형된 부산물을 만들게 된다. AGE는 외인적 또는 내인적 기전을 통해 다양하게 형성될 수 있다(Figure 1). 혈당 상승은 Schiff base의 형성을 유발하고 이것은 결과적으로 더욱 안정한 Amadori product를 형성하게 된다. 이러한 상승은 가역적이며, Amadori product의 농도는 혈당의 수준과 밀접한 관련을 가지고 있다. 일단 Amadori product가 형성되면 이 물질의 산화적 변형과정이 일어나서 비가역적인 AGE가 생성되게 된다. 최종 당화산물의 화학적 구조는 다양하지만 체내에서 발견되는 최종 당화산물의 주된 형태는 carboxymethyllysine-protein 결합이다. 최종 당화산물은 수용체 매개 및 비수용체 의존적인 기전으로 구분되는 다양한 기전에 의해 죽상경화증의 발생을 촉진시킨다. 당화 부산물이 인슐린 저항성에 영향을 미치는 여러 기전들이 있다(Figure 2). 당화 부산물을 매개한 TNF-α의 생성은 직접적으로 인슐린 신호전달을 억제하는 것으로 알려져 있다. 인슐린 자체의 최종 당화산물 또는 methylglyoxal 변형과정은 인슐린 작용의 큰 장애를 가져온다.
미토콘드리아의 이상 등의 원인으로 reactive oxygen species의 생성증가와 같은 인슐린 작용 이상의 결과들은 최종당화산물의 생성 및 목표 조직에서의 인슐린 저항성을 증폭시킨다. 이런 과정들은 aminoguanidine, pyridoxamine, N-acetyl cysteine, TM2002, AGE receptor antagonist 등의 치료로 AGE 생성을 억제하여 목표 조직에서의 인슐린 저항성을 억제시킬 수 있을 가능성이 있다.

REFERENCE
Glycation and Insulin Resistance
Novel Mechanisms and Unique Targets?
Fei Song, Ann Marie Schmidt

Abstract—Multiple biochemical, metabolic, and signal transduction pathways contribute to insulin resistance. In this review, we present evidence that the posttranslational process of protein glycation may play a role in insulin resistance. The posttranslational modifications, the advanced glycation end products (AGEs), are formed and accumulated by endogenous and exogenous mechanisms. AGEs may contribute to insulin resistance by a variety of mechanisms, including generation of tumor necrosis factor-α direct modification of the insulin molecule, thereby leading to its impaired action, generation of oxidative stress, and impairment of mitochondrial function, as examples. AGEs may stimulate signal transduction via engagement of cellular receptors, such as receptor for AGEs. AGE–receptor for AGE interaction perpetuates AGE formation and cellular stress via induction of inflammation, oxidative stress, and reduction in the expression and activity of the enzyme glyoxalase I that detoxifies the AGE precursor, methylglyoxal. Once set in motion, glycation-promoting mechanisms may stimulate ongoing AGE production and target tissue stresses that reduce insulin responsiveness. Strategies to limit AGE accumulation and action may contribute to the prevention of insulin resistance and its consequences. (Arterioscler Thromb Vasc Biol. 2012;32:1760-1765.)

Key Words: advanced glycation end products ▪ receptors ▪ insulin resistance ▪ type 2 diabetes mellitus

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Glycolytic intermediates are intracellular sources of AGEs. In these more rapid Maillard-type reactions, 3 classes of dicarbonyl compounds may form, which are potent glycating agents, including glyoxal, methylglyoxal (MG), and 3-deoxyglucosone. Among these, the most potent glycating agent is likely MG. MG forms via the conversion of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate and principally reacts with arginine groups to form AGEs such as 5-hydro-5-methylimidazolone and the fluorescent AGE, argpyrimidine.3 MG levels can be regulated via the activity of 2 enzymes, glyoxalase I and II; these enzymes detoxify MG and result in MG conversion to d-lactate.4 When the level of the activity of glyoxalases is impaired, MG levels rise, thereby favoring further AGE production.

In addition to glucose or glycolytic intermediate–derived AGEs, lipid peroxidation may result in the formation of reactive carbonyl compounds capable of interacting with proteins, thereby leading to the production of advanced lipoxidation end products. Of note, CML is one of the advanced lipoxidation end products; CML-modified adducts may form both by glycoxidation and by lipid peroxidation pathways. Furthermore, when MG reacts with lysine residues of proteins, a homolog of CML-AGE may be produced, known as Nε-(carboxyethyl)lysine.5 CML-AGE may also be formed by inflammatory mechanisms driven by the myeloperoxidase pathway.6 Also, natural aging and renal failure,7,8 in the absence of other risk factors such as diabetes mellitus, are associated with endogenous AGE formation and accumulation.

Exogenous sources of AGEs may contribute to AGE formation in vivo. Foods high in fat and those cooked at high temperatures contain AGEs, the most prominent of which is CML-AGE and the AGE precursor, MG.9,10 Furthermore, air pollution–associated fly ash was shown to lead to the generation of AGEs on proteins in fibroblasts exposed to this agent for long periods of time.11 Hence, dietary and environmental
sources of AGEs may represent additional key depots for the formation of these products. When detoxification mechanisms (such as glyoxalases) or clearance mechanisms, such as in advanced renal disease, are suppressed, AGE levels and their consequences may be amplified.

**Glycation Products, Receptors, and Mechanisms to Sustain and Enhance AGE Levels**

One of the main mechanisms by which AGEs exert their pathogenic effects is via interaction with cell surface receptors. There are multiple receptors for AGEs such as receptor for AGE (RAGE), CD68, lectin-like oxidized low-density lipoprotein receptor-1, macrophage scavenger receptors, or receptor for advanced glycation endproducts-1-2-3.

RAGE is a signal transduction receptor for AGEs. These receptors may contribute to AGE formation and accumulation. For example, when AGEs bind to RAGE, signal transduction mechanisms are instigated in a process requiring the cytoplasmic domain of the receptor. A chief outcome of AGE-RAGE signaling is the generation of oxidative stress, largely through the nicotinamide adenine dinucleotide phosphate-oxidase system. Because oxidative stress facilitates AGE formation, one consequence of the interaction of AGE with RAGE is the generation of an environment that favors further AGE production. Thus, it was not surprising that in mice devoid of RAGE, levels of oxidative stress and AGEs were lower compared with wild-type RAGE-expressing animals. It has been shown that in the kidney of diabetic OVE26 mice devoid of RAGE, levels of glyoxalase I mRNA and protein were higher compared with those of RAGE-expressing OVE26 mice. In parallel, levels of MG and AGEs were lower in the RAGE-deficient mice, despite equal degrees of hyperglycemia and glycosylated hemoglobin levels.

The potential roles of RAGE in AGE generation have a further layer of complexity, because RAGE is not solely a receptor for AGEs but a multiligand member of the immunoglobulin superfamily. Thus, beyond AGEs, RAGE also transduces the signals emitted by multiple members of the proinflammatory S100/calgranulin family and high-mobility group box 1. These molecules, at least in part via RAGE, upregulate cytokines, matrix metalloproteinases, and gene expression programs in monocytes/macrophages and lymphocytes that amplify tissue-damaging inflammation in settings such as autoimmunity and AGE production. Inflammatory stress itself may generate oxidative stress; for example, one key consequence of activation of the myeloperoxidase pathway is the generation of CML-AGEs. Hence, ligand-RAGE may trigger oxidative stress and inflammation; once S100/calgranulin- and high-mobility group box 1–bearing inflammatory cells are recruited to the sites of tissue stress, release of these non-AGE ligands may exacerbate oxidative stress and stimulate further AGE formation. Figure 1 summarizes the proposed hypotheses regarding triggers to glycation and consequences of glycation, and amplifying pathways further exacerbate generation and accumulation of AGEs.

Although the earliest studies on AGEs involved their mechanistic links to the pathogenesis of diabetic complications, recent intriguing studies have suggested that glycated products and AGEs may contribute to the pathogenesis of insulin resistance as well. In the section to follow, we will present a summary of data in human subjects suggesting that AGEs may be linked to insulin resistance.

**Glycation and Links to Insulin Resistance: Studies in Human Subjects**

Several studies have reported interesting associations between AGE levels and insulin resistance, even in the absence of diabetes mellitus. Tan et al measured AGE levels, associated inflammatory markers, and insulin resistance by the homeostatic model assessment index or HOMA-IR in 207 healthy subjects without diabetes mellitus. Serum levels of AGEs correlated in a statistically significant manner with HOMA-IR in both male and female subjects. Even after adjustment for age, sex, body mass index, waist circumference, cigarette smoking, adiponectin levels, and markers of oxidative stress and inflammation, AGE levels were an independent correlate of HOMA-IR on multiple regression analysis. Tahara et al reported on 322 nondiabetic Japanese subjects; serum AGE levels correlated with HOMA-IR in these subjects, and after multiple regression analysis, AGEs, along with waist circumference, glycosylated hemoglobin, and triglycerides, were correlated with the degree of insulin resistance. When age-adjusted HOMA-IR levels stratified by AGE tertiles were compared using statistical tests, the authors found trends in both male and female subjects. Sarkar et al tested the potential association between levels of total carbonyl compounds in serum with HOMA-IR levels in type 2 diabetic subjects and found a highly significant correlation between the degree of insulin resistance and carbonyl levels, whereas levels of lipid peroxidation end products and thiobarbituric reactive substances were not significantly correlated with HOMA-IR, perhaps suggesting that pre-AGE compounds rather than the pathophysiological state of oxidative stress alone were required to impact insulin sensitivity. Diamanti-Kandarakis et al studied AGE levels in a group of subjects with polycystic ovary syndrome. Subjects with polycystic ovary syndrome often display insulin resistance. Among the 193 subjects studied, 100 had polycystic ovary syndrome, and the remaining subjects were age- and body mass index–matched control individuals. Subjects with polycystic ovary syndrome had significantly higher AGE levels than subjects with isolated hyperandrogenemia, anovulation, ultrasound diagnosed polycystic ovary, and control subjects.

Taken together, these findings in human subjects suggest potential links of AGEs to insulin resistance. Evidence suggestive of mechanistic links between AGEs and insulin resistance was provided by the results of in vitro and in vivo experimentation. In the sections to follow, we discuss these findings and suggest that AGEs might be new therapeutic targets for mitigating insulin resistance.

**Glycation and Insulin Resistance: In Vitro Evidence**

**Direct Effects of Modification of Insulin**

Recent work has suggested that glycation of insulin is a distinct possibility and that such glycation may change the properties of insulin, impacting on its action. Although insulin has a very
short half-life of 5 to 10 minutes, it has been shown that glycation of insulin and proinsulin may occur in the pancreas during the periods of insulin synthesis and storage. Boyd et al prepared monoglycated insulin to correspond to precisely what has been found in vivo, that is, single glycation modification in the phenylalanine position in the amino terminus of the insulin B chain. Compared with nonglycated control insulin, infusion of the glycated form to mice undergoing a glucose tolerance test revealed a 20% reduction in glucose-lowering potency. In isolated abdominal muscle, monoglycated insulin was 20% less effective in mediating glucose update, glucose oxidation, and glycogen production compared with the nonglycated form of insulin.\(^{30}\) In plasma pooled from 4 male human subjects with type 2 diabetes mellitus (mean glycosylated hemoglobin levels of 8.1%), Hunter et al\(^{31}\) showed that monoglycated insulin comprised ≈9% of the total insulin. When pure monoglycated insulin was tested in hyperinsulinemic euglycemic clamp studies, administration of glycated insulin resulted in a reduced requirement for exogenous glucose infusion to maintain euglycemia and revealed that 70% more glycated insulin was required to induce a similar amount of insulin-mediated uptake of glucose.\(^{31}\) Hence, in animal models and human subjects, evidence suggests that glycation of insulin may impair its action.

In distinct studies, Jia et al\(^{32}\) showed that methylglyoxal modification of insulin at an arginine residue of the insulin B chain reduced glucose uptake induced by MG-modified insulin in 3T3-L1 adipocytes and L8 skeletal muscle cells compared with native insulin. In other studies, MG modification of insulin affected insulin signaling directly. Fiory et al\(^{33}\) showed that MG modification of insulin blocked insulin receptor substrate tyrosine phosphorylation and phosphoinositide 3-kinase protein activation in the INS-1 pancreatic \(\beta\)-cell line. Oliveira et al\(^{34}\) showed that MG modification of insulin reduced insulin fibril formation and led to the generation of insulin native-like aggregates. The authors speculated that this would result in diminished insulin action. It is important to note that the extent to which insulin is modified in vivo by MG modification in human subjects is not completely clarified.
Glycation of Distinct Proteins and Insulin Resistance

There are numerous published examples of protein glycation and mechanisms by which they may impact insulin sensitivity. Glycation of albumin has been shown to increase the production of tumor necrosis factor-α. Tumor necrosis factor-α has been linked to insulin resistance via induction of proinflammatory mechanisms that suppress insulin signal transduction.\textsuperscript{35,36} In L6 skeletal muscle myotubes, Cassee et al\textsuperscript{37} showed that human glycated albumin induced Src-mediated activation of protein kinase C-α and suppressed IRS-1 action in a RAGE-dependent manner.

Because of the diverse nature of potential targets for AGE and MG modification, Gugliucci\textsuperscript{38} proposed the hypothesis that MG modification of AMP kinase (AMPK) might contribute to metabolic dysfunction and hepatic insulin resistance. Gugliucci reasoned that the sensing of AMP levels by AMPK is dependent on a domain with 3 arginine residues. If any or all of those residues were modified by the potent glycating agent MG, then it is plausible that functional modification might ensue as well.\textsuperscript{38} Hence, if AMPK activity were reduced, the consequences might include enhanced gluconeogenesis and lipogenesis, both features that characterize hepatic insulin resistance. Although not proved experimentally at this time, the concept nevertheless raises the intriguing possibility that glycated modification of distinct biochemical moieties that contribute to regulation of insulin sensitivity beyond AMPK, such as other key metabolic enzymes, molecules involved in mitochondrial biogenesis, and molecules that modulate oxidative stress, for example, might drive further AGE- or MG-mediated microenvironments conducive to the development of insulin resistance. In this context, efforts to reduce glycation in vivo have been tested in experimental animal model systems.

Glycation and Insulin Resistance: In Vivo Evidence

Support for roles of glycation in vivo has been sought with the use of antiglycating agents to address the question, does reduction or prevention of glycation in vivo block the development of insulin resistance in vulnerable organisms? To test this concept, investigators have tested such agents in an array of animal models.

Unoki-Kubota et al\textsuperscript{39} examined KK-A(y) mice, a mouse model of obesity and type 2 diabetes mellitus, and found that serum levels of AGEs correlated positively with the levels of insulin in these animals. To address whether glycation products played roles in the insulin resistance phenotype, they administered pyridoxamine, an inhibitor of AGE formation, to mice and found that the agent decreased fasting insulin levels and improved insulin sensitivity in a dose-dependent manner, but did not affect fasting blood glucose levels (at least over the short time course of administration).\textsuperscript{39}

Guo et al\textsuperscript{40} treated Sprague-Dawley rats with MG in the drinking water to address the question of whether MG would induce insulin resistance. Two different therapeutic approaches were added to the experimental design: MG alone versus MG+N-acetyl cysteine (an antioxidant) or MG+TM2002, an inhibitor of AGEs. The animals were treated for 4 weeks and then subjected to hyperinsulinemic euglycemic clamp studies. MG administration induced insulin resistance; compared with MG alone, treatment with either N-acetyl cysteine or TM2002 completely improved the insulin resistance induced by MG. In an additional set of animals, co-treatment with MG and high-salt diet was tested. Compared with MG or high-salt diet alone, co treatment resulted in higher systolic blood pressure, increased urinary excretion of albumin and urinary thio-barbituric reactive substances, and renal nitrotyrosine levels. Of note, the AGE derived from MG, Ne-(carboxyethyl)lysine AGE, was higher in any groups treated with MG versus groups not given MG in this study.\textsuperscript{40} Importantly, although these studies did not detail the precise molecular mechanisms by which MG induced insulin resistance, they nevertheless provided evidence linking MG to insulin resistance in vivo.

Kooptiwut et al\textsuperscript{41} addressed these concepts directly in pancreatic islets. They retrieved islets from the pancreata of DBA/2 and C57BL/6 mice and exposed them to 11.1 or 40 mmol/L glucose concentrations alone or in the presence of the AGE inhibitor aminoguanidine. They found that chronic exposure to hyperglycemia resulted in decreased glucose-stimulated (20 mmol/L) islet secretion of insulin in the DBA/2 but not C57BL/6 islets and that this was associated with reduced glucokinase in the DBA/2 islets. When the islets were coincubated with aminoguanidine, the levels of DBA/2 glucokinase rose, thereby suggesting that chronic glucose-mediated increases in AGEs could participate in islet dysfunction over this time course, especially in DBA/2 background, and that efforts to reduce AGEs might be protective.\textsuperscript{41}

Finally, Matsumoto et al\textsuperscript{42} reported on a model system in silkworm in which exposure of the silkworm to high glucose resulted in decreased growth, in parallel with increased AGE levels. When the silkworms were treated with high glucose and aminoguanidine, growth was restored, thereby suggesting that the accumulation of AGEs was contributing to growth suppression. Interestingly, in this model, treatment with 5-aminoimidazole-4-carboxamide riboside (stimulator of AMPK) or insulin also improved growth, thus identifying positive controls for a novel model system to test the adverse effects of high glucose and AGEs on silkworm growth.\textsuperscript{42}

Perspectives, Challenges, and Future Directions

Taken together, the data discussed in this review, from both in vitro and in vivo experimentation, provide supporting evidence that AGEs may contribute, at least in part, to the pathogenesis of insulin resistance by a variety of potential mechanisms, as summarized in Figure 2. The finding that AGE inhibitors, although they may have additional mechanism(s) of action beyond suppressing AGE, suppress insulin resistance in animal models identifies an entirely novel area of therapeutic possibilities for this disorder. It is important to note that in addition to endogenous formation of AGEs, high-fat diet induced AGE introduction in the diet, and environmental pollutants may be linked to AGE generation as well. Hence, dietary modification and reduction in environmental pollutants may also hold...
promise for therapeutic intervention in this key area of public health concern.

It is important to note that recent studies have highlighted potentially adaptive roles for MG in the nervous system. Distler et al.\(^4\) showed that MG is protective against anxiety; the authors showed that glyoxalase 1 increases anxiety in transgenic mice overexpressing this enzyme by reducing GABA\(_A\) receptor activation by MG. Consistent with specific roles for MG, administration of MG assuaged anxiety in the glyoxalase 1 transgenic mice.\(^4\)

Furthermore, data suggest that blockade of cellular receptors for AGEs might be beneficial in insulin resistance states, in which even fleeting bouts of hyperglycemia might facilitate AGE formation sufficient to trigger activation of signal transduction receptors. A clear challenge in the field will thus be to identify the specific AGEs and their levels in plasma and tissues that mediate insulin resistance versus those that trigger diabetic complications. It will be important to decipher whether differential effects of distinct types of AGEs, their levels in the tissue microenvironment, and possible receptor interactions differ in these settings.

Finally, the role of AGE–receptor interaction might be critical to address in therapeutic strategies for additional reasons. Specifically, AGE–receptor interaction has been implicated in the late stage steps in insulin resistance in which accruing damage to the pancreatic \(\beta\)-cell eventually overwhelms the capacity of the islet to compensate for such stress, thereby resulting in hyperglycemia and diabetes mellitus. AGEs have been shown to damage pancreatic \(\beta\)-cells via oxidative stress in a process that appears to require RAGE.\(^4,4\) To what extent AGE precursors and AGEs are implicated in the development of insulin resistance and type 2 diabetes mellitus in vivo is an important question and one worthy of investigation. As the incidence of obesity and insulin resistance continues to rise in adolescents and adults at staggering levels, new approaches to tackling this worldwide epidemic are warranted.

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**References**
