

REVIEW PAPER: CME

Advanced Glycation End Product Cross-Linking: Pathophysiologic Role and Therapeutic Target in Cardiovascular Disease

Advanced glycation end products (AGEs) form by a nonenzymatic reaction between reducing sugars and biological proteins. These stable compounds accumulate slowly throughout the life span and contribute to structural and physiologic changes in the cardiovascular system such as increased vascular and myocardial stiffness, endothelial dysfunction, altered vascular injury responses, and atherosclerotic plaque formation. Mechanisms underlying these alterations include AGE cross-linking of collagen and AGE interactions with circulating proteins and AGE receptors. The clinical manifestations of AGE accrual—isolated systolic hypertension, endothelial and diastolic dysfunction, and atherosclerosis—underscore their role in increased cardiovascular risk associated with aging as well as diabetes and hypertension, conditions that enhance AGE formation. New pharmacologic agents that prevent AGE, break cross-links, or block AGE receptors reduce vascular and myocardial stiffness, inhibit atherosclerotic plaque formation, and improve endothelial function. These agents promise to reduce the risk of isolated systolic hypertension, diastolic dysfunction, and diabetes, and thus, heart failure. (CHF. 2004;10:144–151) ©2004 CHF, Inc.

The Maillard reaction, or browning reaction, was first described in the early 1900s when the French chemist that the condition is named for observed that a yellow-brown pigment formed when he incubated proteins in a glucose solution.¹ This nonenzymatic protein glycation proved to be a key reaction in the food industry, where it underlies the appealing aroma, texture, and coloring of foods. Maillard posited that these glycated proteins go on to form irreversible protein–protein cross-links, the slow accrual of which may play an important role in the pathologic complications of diabetes mellitus. Approximately 90 years later, evidence has accumulated supporting Maillard's conjecture for the pathologic effects of cross-linked glycated proteins not only in diabetes, but also in cardiovascular syndromes, including hypertension, atherosclerosis, and diastolic dysfunction.

Moreover, advanced glycation end products (AGEs) provide a new target for therapeutic agents that prevent or break these cross-links.² This paper reviews the chemical formation of AGEs; their structural, functional, and clinical sequelae in the cardiovascular system; and the potential role of AGE inhibitors in reducing cardiovascular risk.

Formation of AGEs

The Maillard reaction begins with the nonenzymatic attachment of a reducing sugar to a biological amine from a protein, lipid, or amino acid to form a Schiff base (Figure 1). This reaction is swift, highly reversible, and dependent on the concentration of available sugars; lowering the sugar concentration leads to degradation of the unstable base.^{3,4} A Schiff base can undergo further rearrangement to form

a more stable glycated protein known as an Amadori product. As the forward reaction is favored, Amadori products accumulate over time and can undergo additional complex rearrangements to form irreversible cross-links between proteins resulting in AGEs.⁴ Glycation of hemoglobin A_{1c} has served as a useful clinical marker for glycemic control in persons with diabetes. However, AGE accumulation on long-lived proteins such as collagen and lens crystallin has detrimental cardiovascular effects and contributes to cataract formation.^{5–7} AGEs disrupt the structural integrity of proteins and can alter their interaction with circulating proteins and with AGE receptors (RAGEs).^{8,9} In the cardiovascular system, AGE accumulation contributes to arterial stiffening, myocardial relaxation abnormalities, atherosclerotic plaque formation, and endothelial dysfunction (Table I).

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Structural and Physiologic Effects of AGE in the Cardiovascular System

Vascular AGE. Structural Changes and Functional Effects. The strength and elasticity of the vascular wall is primarily derived from the architectural scaffolding of collagen and elastin fibrils. Yet, the longevity of these protein molecules renders them susceptible to cross-linking. AGE-linked collagen is resistant to hydrolytic turnover and accumulates in the vessel matrix in an unorganized, dysfunctional pattern.^{5,10} Elastin molecules are also prone to AGE cross-linking (albeit less so). In vitro, cross-linked elastin has a higher spring constant, but the in vivo vascular consequences of AGE on elastin are unclear.¹¹

AGEs also alter vascular biology primarily by affecting endothelial modulation of vasomotor tone, platelet adhesion and aggregation, thrombogenicity, and cell proliferation. Vlassara et al.¹² demonstrated that AGE deposition is associated with endothelial dysfunction. AGE-modified albumin administered to normotensive, nondiabetic rats or rabbits led to increased vascular permeability, impaired vasodilation to acetylcholine, and mononuclear cell migration, all associated with significantly elevated levels of aortic AGE. These abnormalities were blunted in animals cotreated with aminoguanidine, an AGE inhibitor. AGEs also quench nitric oxide, a key regulatory molecule in endothelial function,¹³

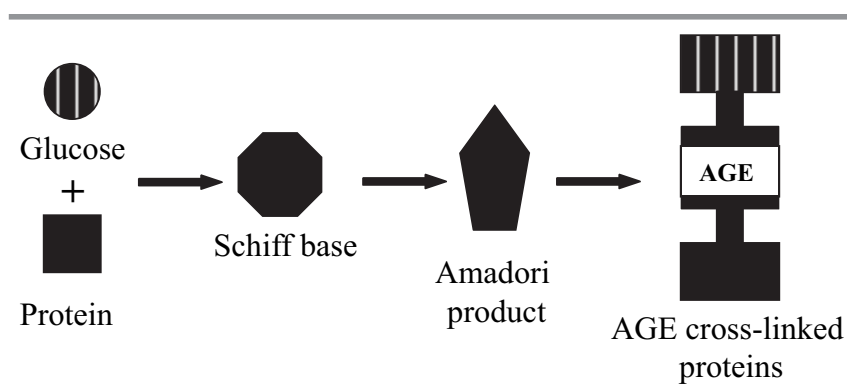


Figure 1. Nonenzymatic protein glycation to form irreversible advanced glycation end product (AGE) cross-linked proteins

and increase the generation of oxidant species such as peroxynitrate.

Another mechanism underlying adverse vascular effects of AGE is via their interaction with their specific receptors.^{9,14,15} RAGEs are members of the immunoglobulin superfamily and are expressed on macrophages, lymphocytes, and endothelial cells.¹⁶ The AGE–RAGE interaction can modulate inflammatory responses by increasing the expression of p12(ras), NF-κB, oxidant radical formation, proinflammatory cytokines and growth factors (e.g., interleukin-6, tumor necrosis factor α, and tissue growth factor β-1) and vascular adhesion molecules.^{17,18} AGE–RAGE interaction also upregulates connective tissue growth factor, cyclooxygenase-2 and PGE-synthase-1, potent inducers of extracellular matrix synthesis, plaque formation, and angiogenesis in diabetes.^{19,20} In response to vascular endothelial injury,

RAGE is upregulated and plays a key role in neointimal hyperplasia.^{14,15}

AGEs also modify vascular function by interaction with circulating proteins that impact cell signaling, vascular permeability, and injury response.² For example, AGE alteration of lipoproteins likely plays an important role in atherosclerosis.

Physiological Implications: Hypertension and Endothelial Function.

Accumulation of vascular AGE alters arterial wall structure leading to increased intimal medial thickening; amassed cross-linked collagen; and frayed, broken elastin fibrils. Clinically, such changes diminish large vessel compliance elevating systolic blood pressure and pulse pressure (PP), while often lowering diastolic blood pressure. Central arterial stiffening poses significant risk for stroke, myocardial infarction, heart failure, and overall mortality.^{21,22} Furthermore, the

Table 1. Advanced Glycation End Product (AGE) Effects in the Cardiovascular System

SITE	AGE TARGET	MECHANISM	PHYSIOLOGIC CHANGE	CLINICAL MANIFESTATION
Vasculature	Collagen Elastin	Cross-linking	Arterial stiffness	Isolated hypertension
	Endothelial cells Macrophages Lymphocytes	RAGE	↓ Vasodilation ↓ Vascular permeability and injury repair ↓ Mononuclear migration, and platelet adhesion ↓ Cell signaling	Endothelial dysfunction, arterial restenosis, atherosclerosis
	Circulating proteins	Cross-linking	↓ Cell signaling and vascular permeability	Vascular injury repair
	Low-density lipoprotein	Cross-linking	↑ Uptake by macrophages	Atherosclerosis
Myocardium	Collagen	Cross-linking RAGE	Myocardial stiffness Calcium signaling	Diastolic dysfunction/ heart failure
RAGE=advanced glycation end product receptor				

physiologic repercussions of vascular stiffening (left ventricular [LV] hypertrophy, decreased coronary perfusion, diastolic dysfunction, and heart failure) can lower the threshold for ischemia, dyspnea, and exercise tolerance. Available antihypertensive agents, which principally lower peripheral vasomotor tone, can exacerbate orthostatic hypotension and cause falls in patients with central vascular stiffening. Therapeutic agents targeted at AGE-induced arterial stiffening may modify structural components of vessels, a process previously considered irreversible. Moreover, interfering with the AGE-modulated endothelial dysfunction may also diminish the risk of atherosclerotic disease.

Myocardial AGE. Structural Changes and Functional Effects. Collagen in the myocardial matrix is also subject to AGE cross-linking leading to increased myocardial stiffness.²³ With increasing age, myocardial collagen concentration and the percentage of AGE cross-linked collagen fibrils increases.⁷ Myocardial collagen is also susceptible to AGE cross-linking during times of increased collagen deposition, such as in LV hypertrophy in response to ventricular volume²⁴ and/or pressure overload.²⁵ The influence of AGE in the heart is on both passive and active diastolic properties. Passive changes are related principally to AGE-induced myocardial stiffening, whereas active relaxation is also altered by AGE-RAGE modification of calcium signaling.^{23,26}

Physiological Implications: Diastolic Dysfunction. Impairment of early LV filling, or diastolic dysfunction, contributes to impaired exercise tolerance, exertional dyspnea, and a reduced ischemic threshold.²⁷ As with isolated systolic hypertension, the prevalence of diastolic heart failure increases with age and is associated with elevated morbidity and mortality. AGE cross-linking of myocardial collagen fibrils is associated with diastolic dysfunction and the administration of AGE inhibitors has

been shown to prevent ventricular stiffening, thus revealing another potential target of these therapies.^{28,29}

AGE and Atherosclerosis. Another AGE target with cardiovascular repercussions is modification of low-density lipoprotein (LDL). Researchers have demonstrated that AGE formation on the apoprotein component of LDL increases its susceptibility to cross-linking with the collagen fraction of the vessel wall, reducing the uptake of LDL by its receptors.³⁰⁻³² This increases serum LDL levels that are more prone to oxidation and uptake by macrophages to form foam cells, an integral component of atherosclerotic plaques. Cross-linking collagen fibrils with other circulating proteins can also alter the dynamic integrity and function of the vascular matrix, affecting tissue repair mechanisms and contributing to atherosclerotic plaque formation.^{2,5,8}

AGE in Diabetes and Hypertension. The structural and functional consequences of AGE accumulation in the cardiovascular system are exaggerated in patients with diabetes and hypertension.⁴ In persons with diabetes, the high glucose concentration accelerates AGE cross-linking in the vasculature, leading to increased arterial stiffness.^{4,33} Likewise, the propensity of persons with diabetes to develop premature cardiovascular disease and to experience higher rates of restenosis following percutaneous coronary interventions are, in part, explained by AGE effects on vascular stiffening, tissue repair, impaired cellular signaling, atherosclerotic plaque formation, and myocardial stiffening.^{3,4,20,34} Collagen cross-linking is also accelerated in states of high vascular collagen synthesis, as with increased mechanical stress as in hypertension.²⁵

Therapeutic Agents Targeted to AGE

Three general classes of drugs exist that target AGE pathophysiology: agents that inhibit de novo AGE synthesis, drugs that break preexisting

AGE cross-links, and truncated soluble RAGEs that serve as receptor blockers. Figure 2 summarizes the targets for these agents and Table II summarizes the therapeutic status.

Inhibitors of AGE Synthesis. In the mid 1980s, Ulrich and Cerami² developed aminoguanidine (AG), a carbonyl reagent that reacts with ketones rendering them chemically inert to glycation and AGE formation. When administered long-term to normotensive, nondiabetic rats, AG reduced age-associated arterial stiffness compared with placebo-treated littermates.^{28,35} Similar reductions in vascular stiffness were seen in diabetic rats treated with AG compared with those treated with placebo; AG-treated animals had reduced aortic AGE and cross-linked collagen and decreased PP and characteristic impedance and increased carotid compliance.^{33,36} In the myocardium, AG reduced age-associated hypertrophy when administered to normotensive rats.²⁸ Because the amounts of myocardial and vascular wall collagen and elastin were unaltered by AG treatment, the authors concluded that reduced afterload due to decreased aortic collagen cross-linking blunted the age-related hypertrophic response. AG also reduced aortic atherosclerotic plaque formation by 30%–48% compared with placebo in nondiabetic rabbits fed a high-cholesterol diet.³⁷ This marked plaque reduction was independent of hypolipidemia or antioxidant effects. While these experimental results have been intriguing, clinical trials of AG are limited by adverse effects, including gastrointestinal symptoms, liver abnormalities, and renal vasculitis. Two other agents have been developed that inhibit AGE synthesis: pyridoxamine and \pm 2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195). The latter agent reduces systolic blood pressure in spontaneously hypertensive rats, decreasing the oxidative state and increasing endothelial nitric oxide gene expression.³⁸ In nondiabetic rats, OPB-9195 inhibited intimal thickening after balloon injury in the carotid artery.³⁹

AGE Cross-Link Breakers. Continued efforts to develop new therapeutic agents to prevent the formation of AGE led to the surprising discovery of a new class of drugs that could actually break preexisting AGE cross-links.⁴⁰ The ability of the thiazolium portion of this new agent to cleave AGE cross-links translated to the first agent to potentially reverse the pathologic effects of AGE.⁴¹ Subsequent work to develop a more active and stable compound led to the development of 4,5-dimethyl-3-phenylacetylthiazolium chloride, an AGE cross-link breaker, and more recently, 4,5-dimethyl-3-(2-oxo-2phenylethyl)-thiazolium chloride (ALT-711).

In animal models, AGE cross-link breakers reduce aging and diabetic-associated vascular stiffness. When administered to streptozotocin-induced diabetic rats, ALT-711 increased carotid compliance and distensibility and systemic vascular compliance and decreased aortic characteristic impedance compared with placebo-treated diabetic animals.⁴² These changes were independent of serum glucose levels, were associated with decreased red blood cell-IgG cross-links, and were greater as rats were treated for longer. Candido et al.⁴³ examined the effect

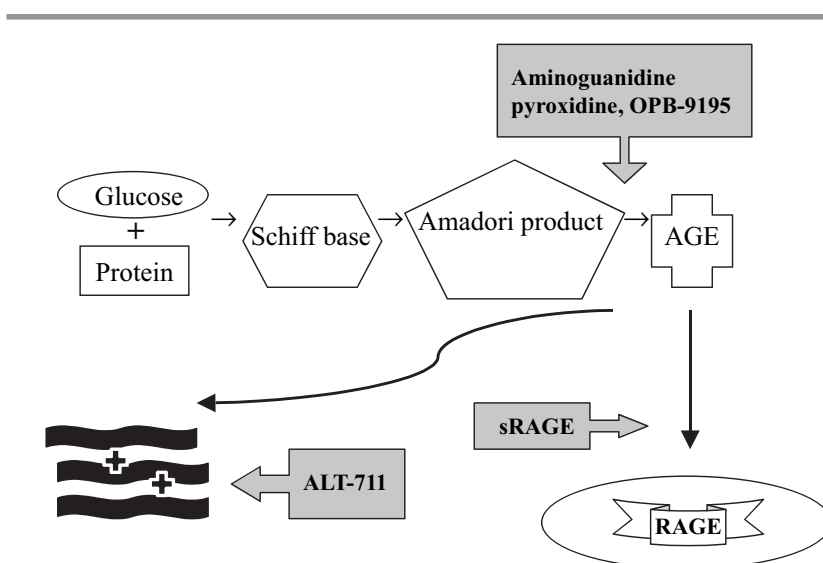


Figure 2. Sites of action of advanced glycation end product (AGE) inhibitors. OPB-9195 = \pm 2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide; ALT-711 = 4,5-dimethyl-3-(2-oxo-2phenylethyl)-thiazolium chloride; sRAGE = soluble AGE receptor; RAGE = AGE receptor

of AGE on diabetes-related vascular and ventricular stiffening. In contrast to their nondiabetic littermates, diabetic rats had increased cardiac AGE, decreased ventricular collagen solubility due to more cross-linking, increased collagen III molecules, augmented RAGE expression, and increased connective tissue growth factor and cardiac brain natriuretic peptide gene

expression. These changes were not observed when the diabetic rats were treated with ALT-711. Moreover, ALT-711 restored collagen solubility to that of nondiabetic controls, implying the agent indeed acted by reducing collagen cross-linking.

AGE cross-link breakers also reduce ventricular stiffening associated with aging and diabetes. In nondiabetic older

Table II. Status of Advanced Glycation End Product (AGE)-Targeted Therapeutic Agents*

SOURCE	AGE INHIBITORS			AGE CROSS-LINK BREAKERS	
	AMINO GUANIDINE	PYRIDOXAMINE	OPB-9195	ALT-711	RAGE INHIBITORS sRAGE
Animal studies	↓ Arterial stiffness ↓ LV stiffness ↓ Atherosclerotic plaques	↓ Atherosclerotic plaques	↓ Vascular injury response ↓ Systolic blood pressure	↓ Arterial stiffness ↓ LV stiffness	↓ Atherosclerotic plaques ↓ Vascular injury response
Clinical trials	↓ Diabetic nephropathy ⁵¹ study stopped early—limiting side effects: GI, liver abnormalities, renal vasculitis	None	None	↓ Vascular stiffness ↓ LV mass/↑ diastolic compliance Ongoing: ISH, ISH with LVH	None
RAGE=advanced glycation end product receptor; OPB-9195= \pm 2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide; ALT-711=4,5-dimethyl-3-(2-oxo-2phenylethyl)-thiazolium chloride; sRAGE=soluble RAGE; LV=left ventricular; GI=gastrointestinal; ISH=isolated systolic hypertension; LVH=left ventricular hypertrophy; *potential clinical targets include diabetic complications (vascular, nephropathy, retinopathy), atherosclerotic disease, vascular injury response/restenosis, ISH, diastolic heart failure, Alzheimer's disease, aging skin changes, cataract formation, and degenerative nerve disorders.					

dogs, ALT-711 treatment reduced LV stiffness by 40% indexed by increased end diastolic volume without pressure change, creating an improvement in stroke volume.⁴⁴ Likewise, ALT-711 decreases arterial and myocardial stiffness in aged nondiabetic primates optimizing ventricular-vascular coupling, an important property for exercise capacity in older adults.⁴⁵ Significant increases in vascular compliance (decreased pulse wave velocity and augmentation index) and reduction in myocardial stiffness (increased LV end diastolic dimension, stroke volume, and fractional shortening), were seen after ALT-711 treatment. Vascular and myocardial stiffening reappeared after discontinuation of the drug. Recent data in older diabetic dogs have revealed improved LV systolic function and aortic compliance in animals treated with ALT-711. Myocardial collagen type I and II protein content, which was upregulated by diabetes, was reduced by ALT-711, and collagen solubility was restored.⁴⁶

Clinical studies to date have shown ALT-711 to be well tolerated with a safety profile similar to placebo. In a randomized, placebo-controlled trial of 93 subjects older than age 50 years with vascular stiffness (PP > 60 mm Hg or isolated systolic hypertension), total arterial compliance increased 15%–20% while arterial PP declined with oral ALT-711 treatment.⁴⁷ A recent

open-label trial²⁹ of patients with heart failure with preserved ejection fraction found ALT-711 reduced LV mass by 5%, and enhanced early diastolic filling and patient-reported symptoms. Results of phase 2A studies of ALT-711 in systolic hypertension with and without ventricular hypertrophy (Systolic And Pulse Pressure Hemodynamic Improvement by Restoring Elasticity and Systolic Hypertension Interaction with Left VEntricular Remodeling trials, respectively) are to be released in summer 2004.

AGE Receptor Blockers. Another strategy to inhibit AGE pathophysiology has been targeting RAGE. RAGE blockade has been achieved by infusible soluble RAGE (sRAGE), which acts as a false ligand, and by RAGE antibodies. sRAGE improves wound healing in diabetic mice, suppresses early acceleration of atherosclerosis, and stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice.^{48,49} Animals treated with sRAGE demonstrate reduced expression of vascular adhesion molecules-1, tissue factor, macrophage chemotactic protein-1, and matrix metalloproteinases.⁵⁰ In mice, sRAGE significantly decreased endothelial tissue injury, including neointimal hyperplasia, smooth muscle proliferation, extracellular matrix molecule expression, and cellular migration.¹⁴ Similar reductions in vascular

smooth muscle cell proliferation and neointimal formation following balloon injury occur in diabetic rats treated with sRAGE compared with nondiabetic rats.¹⁵ There are presently no small molecule inhibitors of RAGE, although this is under development, and this approach has yet to be tested in clinical studies. Nonetheless, these experimental data support this strategy.

Summary and Future Directions

Ninety years after Maillard first described protein glycation, appreciation for the clinical pathophysiologic importance of this reaction is growing rapidly. AGE has structural and functional cardiovascular effects that worsen the risk of heart and arterial disease, particularly in persons with diabetes, but also as a component of aging and hypertension. Although AGE-related changes were first considered irreversible, recent studies suggest this is not the case. Newer pharmaceutical agents that break AGE cross-linking appear to hold promise and ongoing studies may define their clinical utility.

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CME Questions

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INSTRUCTIONS FOR COMPLETING THIS FORM: Read the selected paper and answer *all* the questions that follow. After each question there is a series of possibly correct answers. Please select the one best answer for each and place your selection on the answer grid. **YOU MUST ALSO COMPLETE THE CME EVALUATION SECTION** and return the form within 6 months of the paper's publication to receive credit. Letters of credit will be mailed to participants biannually.

ACCREDITATION STATEMENT: Winthrop-University Hospital (WUH) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education for physicians. WUH designates this Continuing Medical Education activity for a maximum of (1) credit hour in Category 1 credit towards the AMA Physicians' Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity. WUH relies upon faculty participants in its CME programs to provide educational information that is objective and as free of bias as possible. In this spirit, and in accordance with the guidelines of the program sponsor, faculty participants are expected to indicate any commercial relationship that might be perceived as a real or apparent conflict of interest.

OBJECTIVE AND TARGET AUDIENCE: All health care providers are eligible to receive credit. At the conclusion of this activity, participants should be able to: 1) summarize the important points discussed in the paper reviewed; 2) identify patients to whom the paper is relevant; 3) modify management practices as new information is learned; and 4) identify deficiencies in their knowledge base.

Please Select the One Best Answer for Each and Place Your Selection on the Answer Grid.

1. Which of the following is not the result of accumulated advanced glycation end products (AGEs)?
 - A. Endothelial dysfunction
 - B. Atherosclerotic plaque formation
 - C. Vascular laxity
 - D. Altered vascular injury response
2. One mechanism by which AGEs cause alteration to the cardiovascular status is:
 - A. Endothelial proliferation
 - B. Reduction in circulating proteins
 - C. Alteration of electrical conductivity
 - D. Cross-linking of collagen
3. Of the following, which is not a physiologic target of AGEs?
 - A. Lymphocytes
 - B. Melanocytes
 - C. Collagen/elastin
 - D. Macrophages
4. Therapeutic agents targeted at AGEs act by:
 - A. Blocking AGE receptors
 - B. Inhibiting AGE mutations
 - C. Supporting AGE cross-links
 - D. Lowering peripheral vasomotor tone
5. In animal models, the thiazolium portion of investigational agents was shown to reduce all of the following AGE-related effects except:
 - A. Cross-linking
 - B. Aging
 - C. AGE receptor activity
 - D. Diabetes-associated vascular stiffness



CME Answers are available on the *Congestive Heart Failure* page at www.lejacq.com

CME Answer Grid

Answer the questions from the previous page by selecting the best choice of A, B, C, or D.

Questions: 1. __ 2. __ 3. __ 4. __ 5. __

CME Evaluation

	Agree			Disagree
1. My knowledge was enhanced by this activity.	1. __	2. __	3. __	4. __ 5. __
2. The activity helped to clarify issues specific to heart failure patients.	1. __	2. __	3. __	4. __ 5. __
3. The information obtained from this exercise will have an impact on my care of patients.	1. __	2. __	3. __	4. __ 5. __
4. The format of the exercise was useful.	1. __	2. __	3. __	4. __ 5. __
5. Suggestions for future topics:				

Where to Send the Completed CME Form

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Re: Zieman SJ, Kass DA. Advanced glycation end product cross-linking: pathophysiologic role and therapeutic target in cardiovascular disease. *Congest Heart Fail.* 2004;10:144–151.

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