

# The Role of AGEs and AGE Inhibitors in Diabetic Cardiovascular Disease

M.C. Thomas<sup>1,\*</sup>, J.W. Baynes<sup>2</sup>, S.R. Thorpe<sup>2</sup> and M.E. Cooper<sup>1</sup>

<sup>1</sup>*Danielle Alberti Memorial Centre for Diabetes Complications, Baker Medical Research Institute, Melbourne, Victoria, Australia and* <sup>2</sup>*Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC, USA*

**Abstract:** Prolonged hyperglycemia, dyslipidemia and oxidative stress in diabetes result in the production and accumulation of AGEs. It is now clear that AGEs contribute to the development and progression of cardiovascular disease in diabetes, as well as other complications. AGEs are thought to act through receptor-independent and dependent mechanisms to promote vascular damage, fibrosis and inflammation associated with accelerated atherogenesis. As a result, novel therapeutic agents to reduce the accumulation of AGEs in diabetes have gained interest as potential cardioprotective approaches. A variety of agents have been developed which are examined in detail in this review. These include aminoguanidine, ALT-946, pyridoxamine, benfotiamine, OPB-9195, alagebrium chloride, N-phenacylthiazolium bromide and LR-90. In addition, it has been demonstrated that a number of established therapies have the ability to reduce the accumulation of AGEs in diabetes including ACE inhibitors, angiotensin receptor antagonists, metformin, peroxisome proliferators receptor agonists, metal chelators and some antioxidants. The fact that many of these inhibitors of AGEs are effective in experimental models, despite their disparate mechanisms of action, supports the keystone role of AGEs in diabetic vascular damage. Nonetheless, the clinical utility of AGE inhibition remains to be firmly established. Optimal metabolic and blood pressure control, that is achieved early and sustained indefinitely, remains the best recourse for inhibition of AGEs until more specific interventions become a clinical reality.

**Key Words:** Advanced glycation end products (AGEs), Atherosclerosis, Alagebrium, Cardiovascular disease, Diabetes, Dyslipidemia, Receptor for AGEs (RAGE).

## BACKGROUND

Patients with diabetes have a mortality rate from cardiovascular disease (CVD) that is over twice greater than the general population [1]. Macrovascular complications including stroke, myocardial infarction and peripheral vascular disease, develop in more than half of the diabetic population. While classical risk factors for CVD such as smoking, cholesterol and hypertension, operate in persons both with and without diabetes, the absolute risk of death is 2-4 times greater in patients with diabetes and progressively larger with each additional risk factor [2]. Diabetes appears to act as an amplifier of cardiovascular risk, multiplying the clinical impact of the underlying risk factors, leading to the increased incidence, size and complexity of atherosclerotic plaques and an increased prevalence of cardiovascular events. Despite the evident dangers from diabetes, knowledge of the underlying mechanisms contributing to diabetic vascular pathology is limited. Clearly, the fundamental abnormality associated with diabetes is the chronic elevation of circulating glucose levels. Current management practices are based around the paradigm that the best way to reduce the risk for cardiovascular complications is to achieve optimal glycaemic control. Yet, a complete understanding of the manner in which an excess of glucose contributes to kidney failure, blindness, CVD and amputations, remains a major challenge to biomedical research. Although a number of haemodynamic and metabolic factors operate in diabetes,

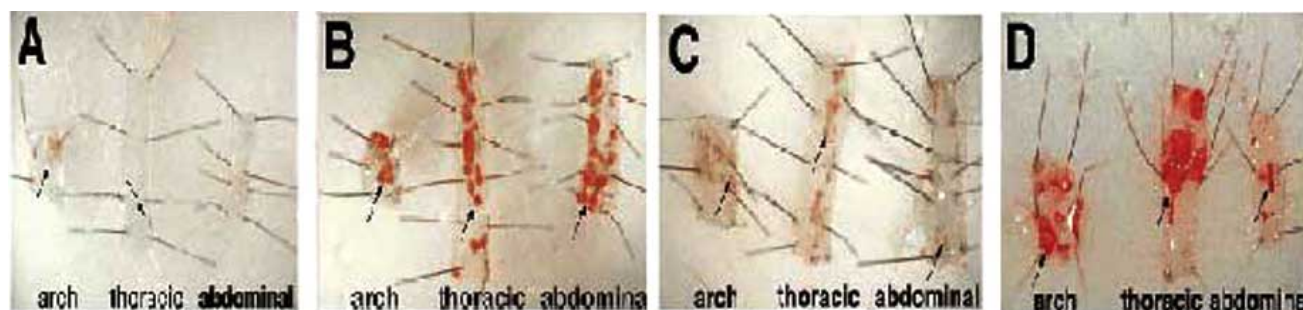
one pivotal pathway appears to be the formation and accumulation of *Advanced Glycation End-products* (AGEs).

Prolonged hyperglycemia, dyslipidemia and oxidative stress in diabetes result in the production and accumulation of AGEs [3] in the kidney [4], the retina [5], in atherosclerotic plaques (illustrated in Fig. (1)) [6] and at other sites of diabetic complications [7]. AGEs have a wide range of chemical, cellular and tissue activities implicated in the development and progression of vascular disease in diabetes [8, 9]. Their importance as downstream mediators of hyperglycemia in diabetes has been amply demonstrated in animal studies using inhibitors of advanced glycation that retarded the development of vascular disease without directly influencing plasma glucose levels [6, 10]. Furthermore, excess dietary AGEs fed to Apolipoprotein E (Apo E) knockout mice have been shown to accelerate atherosclerosis, in the absence of hyperglycemia [11]. This review will examine the role of AGEs in the pathogenesis of cardiovascular complications in diabetes and explore the potential utility of interventions designed to prevent the accumulation of tissue AGEs in diabetes (Table 1).

## WHAT ARE AGEs?

AGEs are formed by non-enzymatic *Maillard* or 'browning' reaction between reducing sugars and amino groups on proteins, lipids or nucleic acids. The first stable adduct between glucose and protein is fructose-lysine or the *Amadori* product. Subsequent dehydration, oxidation, rearrangement and fragmentation reactions lead to the formation of AGEs. However, under physiological conditions (pH 7, 37°C), this reaction is slow, meaning that AGE-

\*Address correspondence to this author at the Baker Medical Research Institute, PO Box 6492, Melbourne, VIC 8008, Australia; Tel: +61-3-85321280; Fax: +61-3-85321480; E-mail: mthomas@baker.edu.au



**Fig. (1).** Sudan IV staining of plaques [6] showing accelerated atherosclerosis in apoE diabetic mice (B) compared to control (A) and attenuation of plaque development in mice treated with an AT1 antagonist – irbesartan (C) but not amlodipine (D).

**Table 1. Potential Inhibitors of AGE Accumulation**

Aminoguanidine
ALT-946 [N-(2-Acetamidoethyl)hydrazinecarboximidamide hydrochloride]
Pyridoxamine
OPB-9195 [(+/-)-2-isopropylidenehydrazono-4-oxo- thiazolidin-5-ylacetanilide]
Pyridoxamine-aminoguanidine conjugate
N-PTB [N-phenacylthiazolium bromide]
ALT-71 [Alagebrium chloride]
LR-90 [Methylene bis [4,4'—(2-chlorophenylureidopheonoxyisobutyric acid)]
Carnosine, Homocarnosine
Antioxidants
Alpha-lipoic acid
ACE inhibitors
Angiotensin receptor antagonists
Metal chelators (including trientine, desferrioxamine, penicillamine)
Metformin
Peroxisome proliferators receptor agonists (PPARs)

modifications accumulate primarily in long-lived molecules such as extracellular matrix proteins such as collagen and crystallins [3]. Over a person's lifespan, the amount and variety of AGE-modified tissue progressively increases, due in part to the time-dependence of the *Maillard* reaction but also reduced protein turnover associated with chronological aging [12], increased cross-linking and the resistance of AGE-modified proteins to proteolytic digestion. AGEs may also contribute to changes associated with aging in the cardiovascular system such as increased vascular and myocardial stiffness. In patients with diabetes, persistent hyperglycemia, dyslipidemia and oxidative stress, all act to hasten the formation of AGEs [13], meaning not only that long-lived proteins become more heavily modified, but also that shorter-lived molecules, such as apolipoproteins and LDL, become targets for progressive AGE-modification [3, 9].

AGEs are a chemically heterogeneous group of compounds, most of them structurally uncharacterized. In addition, the factors that determine the levels and type of AGE-

modification remain to be fully established. The pattern of AGE-products formed in any reaction is critically dependent on the precise conditions in which the reaction is occurring. For example, it may be influenced not just by the reaction on a single amino group and sugar, but also by interactions between *Amadori* products, protein and sugar fragmentation pools [14]. Other factors may also influence the type and quantity of AGEs formed. For instance, time, temperature, pH, traces of transition metal ions, oxygen concentration and endogenous Maillard inhibitors (such as spermine and carnosine) may all result in changes to the observed product ratios [15]. The resulting complex chemistry elaborates a diverse range of heterocycles, polymers and advanced Maillard products (AGEs).

N-(carboxymethyl)lysine (CML), is the simplest and best characterized AGE, derived predominantly from the carbonyl modification of lysine. CML is also the main epitope for recognition by most commercially available antibodies used for the detection and quantification of AGEs [16]. Tissue CML concentrations are increased in aging

tissue [17]. In diabetes [18], elevated CML levels are associated with the presence of vascular complications in patients with diabetes [19].

Other more complex AGEs form 'cross links' both between and within modified proteins, such as pentosidine, MOLD (methylglyoxal lysine dimer) and GOLD (glyoxal lysine dimer), [20]. These cross links have the potential to produce important changes in protein structure and function. For example, the glycation of collagen results in altered packing density [21] and surface charge [22], and manifested by increased stiffness and resistance to digestion by metalloproteinases [23, 24]. An increased number of cross links in diabetic collagen is also reflected in the accumulation of acid-insoluble collagen in diabetic tissues, including the heart and vasculature, leading to early and enhanced cardiac and vascular stiffening in the context of diabetes [25]. Many of these AGE cross link moieties have intrinsic fluorescence. This means that fluorescence can be used as a surrogate marker for the presence of these AGE-modifications. For example, tissue fluorescence at various sites has been shown to increase with chronological aging [26]. With the development of diabetes, there is also a marked increase in fluorescence within the skin and vasculature [27]. Plasma fluorescence also correlates with the severity of renal, retinal and vascular complications in patients with diabetes [28].

Other non-fluorescent AGEs involve modification of arginine residues. In particular, the hydroimidazolone AGEs, derived from glyoxal, methylglyoxal and 3-deoxyglucosone, appear to be quantitatively the most common detectable AGEs, both in diabetic tissue [29] and in the circulation [30]. Some data suggest that hydroimidazolone may be selectively

increased in diabetic tissues over and above other AGEs, including CML and pentosidine [30]. Moreover, there is evidence that proteins modified only by hydroimidazolone adducts are able to induce relevant physiological changes, such as the production of the cytokines interleukin 1 and tumor necrosis factor- in human monocytes and macrophages [31].

Although about twenty-five AGEs have been chemically characterized, the identity of the AGEs contributing to the development of diabetic vascular complications have not been clearly determined. It is possible that these known and commonly-measured AGEs may be a marker for the presence of as-yet-unmeasured toxic AGEs [3]. In addition, although specific AGEs may have pro-inflammatory, pro-carcinogenic and pro-apoptotic effects *in vitro*, it is possible that there is an aggregate effect *in vivo*, rather than an effect dependent on individual structures. Moreover, at least some of AGE-mediated injury in diabetes occurs through the activation of multi-ligand AGE-receptors, that recognize a range of chemical structures and formations [9] (Fig. (2)). Consequently, the precise chemical modification or even their abundance may be of less consequence than their ability to reach, bind and activate AGE receptors and thereby relevant pathogenic pathways.

#### AGEs AND MACROVASCULAR DISEASE

While there are a number of factors to the increased number, size and complexity of atherosclerotic plaques in diabetic patients, AGEs are viewed as having a pivotal role in the initiation and acceleration of atherosclerosis. AGEs have been detected within atherosclerotic lesions in both

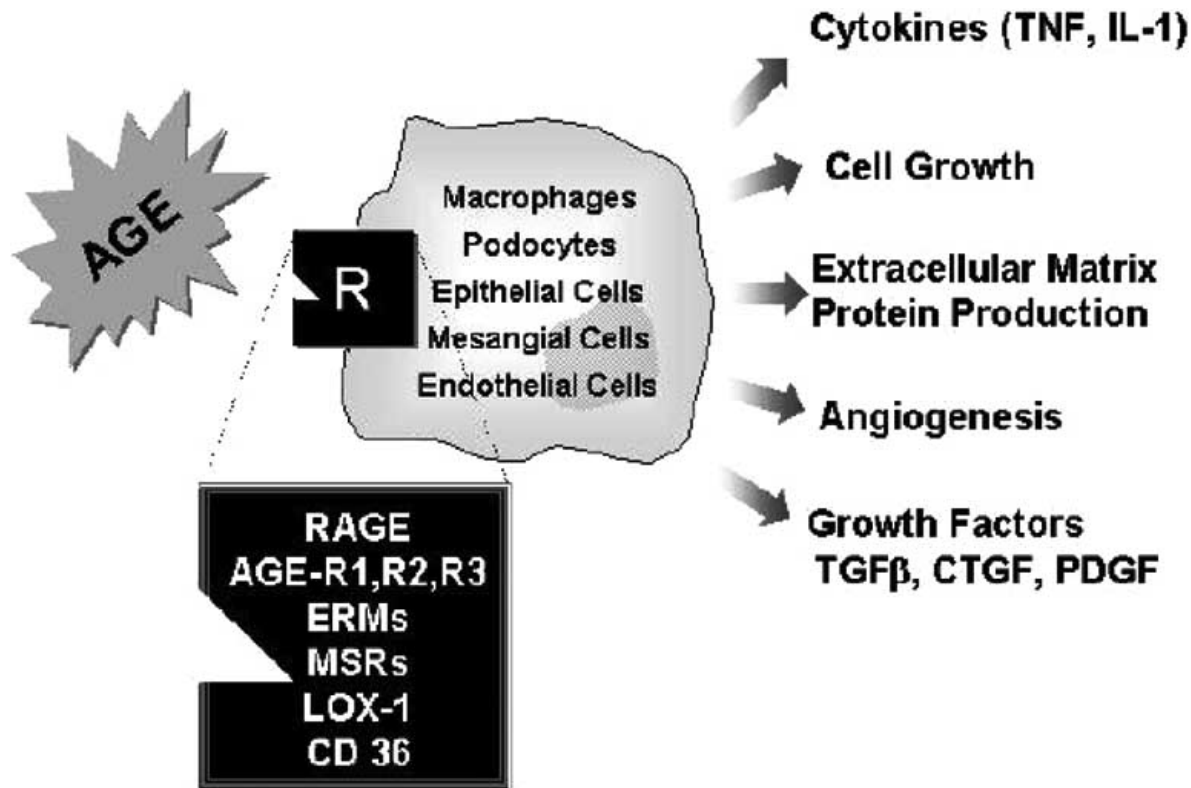


Fig. (2). Cellular responses induced following activation of AGE receptors.

extra- and intra-cellular locations and correlate with the size and complexity of the lesions (Fig. (1) [32]. Clinical studies in patients with type 1 diabetes demonstrate a strong correlation between AGE accumulation and the severity of diabetic complications [33]. For example, in the Diabetes Control and Complications (DCCT) study, AGE levels were overall a better predictor of vascular complications than glycated (*Amadori*) hemoglobin, with over a third of the variance in complications in the study attributed to differences in AGE indices [34]. AGE accumulation in the vessel wall of carotid arteries also correlates with the presence of occlusive disease requiring endarterectomy [35]. In addition, plasma levels of AGEs have been correlated with hypertension and ischemic heart disease, and may be considered as a bio-marker of diabetes-associated cardiovascular risk [36].

### RECEPTOR-INDEPENDENT EFFECTS OF AGEs

AGE-modification of proteins may impact on their structural and functional integrity. This is particularly the case with matrix proteins, because of their slow turnover. For example, the formation of inter- and intramolecular cross links after the glycation of collagen leads to structural alterations, including changes in packing density and surface charge, manifested by increased stiffness, reduced thermal stability and resistance to proteolytic digestion. Cell-matrix interactions may also be disrupted by matrix glycation, contributing to changes in cellular adhesion, altered cell growth and loss of the epithelial phenotype. In addition, heterotypic interactions between matrix proteins and homotypic interactions required for polymeric self-assembly are disturbed by AGE modifications. Furthermore, AGEs have been shown to directly generate reactive oxygen intermediates [37] and quench nitric oxide [38].

When occurring in the vessel wall, such changes ultimately impact on vascular function. For example, the formation of cross links and the accumulation of vascular collagen, reduces the elastic properties of the arterial wall, leading to increased barotrauma of the vessel wall and contributing to the development of systolic hypertension [39]. In addition, large artery stiffening has recently been identified as an important predictor of cardiovascular [40] and coronary [41] mortality, independent of other major risk factors in hypertensive patients and in the elderly [42]. Experimental models of large artery stiffening also impair coronary perfusion [43] and increase myocardial work [44].

Cross links also affect the permeability and charge of the extracellular matrix, which contribute to the irreversible trapping of macromolecules such as lipoproteins in the vessel wall [45]. Such molecular trapping, together with the impaired degradation of AGE-modified proteins, potentially contribute to the accumulation of amorphous hyalinized material that characterizes diabetic vascular disease. Notably vascular hyalinization in diabetes can be attenuated by therapies which modulate vascular AGE accumulation, including direct inhibitors of the AGE formation and Angiotensin Converting Enzyme (ACE) inhibitors [3, 46].

### RECEPTOR-DEPENDENT ACTIONS OF AGEs

Many of the effects of AGEs in diabetes appear to be mediated by interactions with specific AGE-receptors and

binding proteins [9] (Fig. (2)). These receptors are present on various vascular cell types including the endothelium and in mononuclear phagocytes [27]. Over the last decade, a number of specific AGE-receptors and binding sites have been identified. A number of cell-associated AGE-specific receptors and binding proteins have been identified including the receptor for advanced glycation end products (RAGE) [47] AGE-R1 (p60), AGE-R2 (80k-H, protein kinase-C substrate) AGE-R3 (galectin-3) [48], lysozyme [49], as well as the macrophage scavenger receptors (MSR) ScR-II and CD-36 [50]. In addition, we have recently described a novel AGE-binding domain in the ezrin-radixin-moesin family of proteins (ERM-proteins) that are constitutively expressed at high levels in the vasculature, making them good candidates for physiological AGE-interactions [51]. Most of these AGE-binding proteins are constitutively expressed in a limited number of cell types and at low levels in the absence of injury and inflammation. Expression is markedly enhanced in response to metabolic states such as diabetes, dyslipidemia, uremia and aging, possibly due to high levels of AGEs in these conditions.

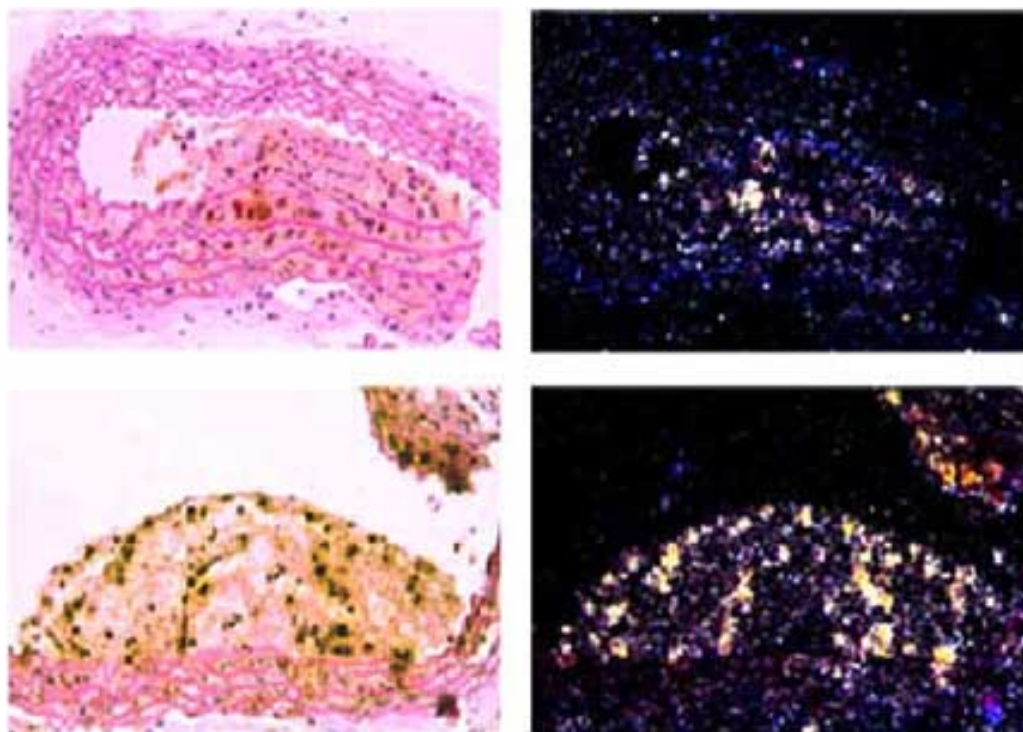
Each of the AGE-receptors is multi-specific, able to bind and be activated by a range of molecules including non-AGE moieties. Indeed, many of the AGE-binding proteins in the body may be accidental AGE-receptors. For example, RAGE has the ability to bind to  $\alpha$ -amyloid protein, several of the S-100 calgranulins, and the neuroregulatory protein, amphoterin [52]. In addition, AGE-receptors have the ability to bind to a wide range of structures. However, it remains to be established which AGEs have the greatest affinity and activating potential for AGE-receptors. AGEs contribute negative charge and hydrophobicity to target proteins, so that pattern recognition of topological features on AGE-modified proteins may contribute to a diffuse recognition process involving a wide range of ligands and scavenger-type receptors. While it is possible that one structural moiety has the greatest pathogenicity, it seems more likely that, given the broad chemical heterogeneity of AGEs, the extent of modification may be more important than the presence of any one chemical distinct AGE [53]. This fact is particularly important when considering many of the studies exploring potential AGE receptors have used heavily-modified exogenous AGEs to trigger events in cell culture, though a similar degree of modification is not seen under normal physiological conditions. In addition, the interpretation of many initial reports were complicated by the presence of bacterial endotoxins which also activate various cell model systems [54]. Consequently, more recent studies have explored the use of endotoxin-free minimally AGE-modified proteins as physiologically relevant ligands for AGE receptors [55] or *in vivo* modified-proteins obtained using affinity chromatography.

The exact role of AGE receptors in normal physiology is still unclear. One view is that AGE-receptors identify senescent tissue elements for excretion or catabolism as well as modulation of cellular properties by receptor-triggered signal transduction on AGE engagement [28, 56]. The specific up regulation of AGE-receptors at sites of injury, such as in diabetic atherosclerotic plaques [57], is also consistent with a role for AGEs in inflammation, cell differentiation and tissue remodeling and repair [8, 9]. However, it is now established

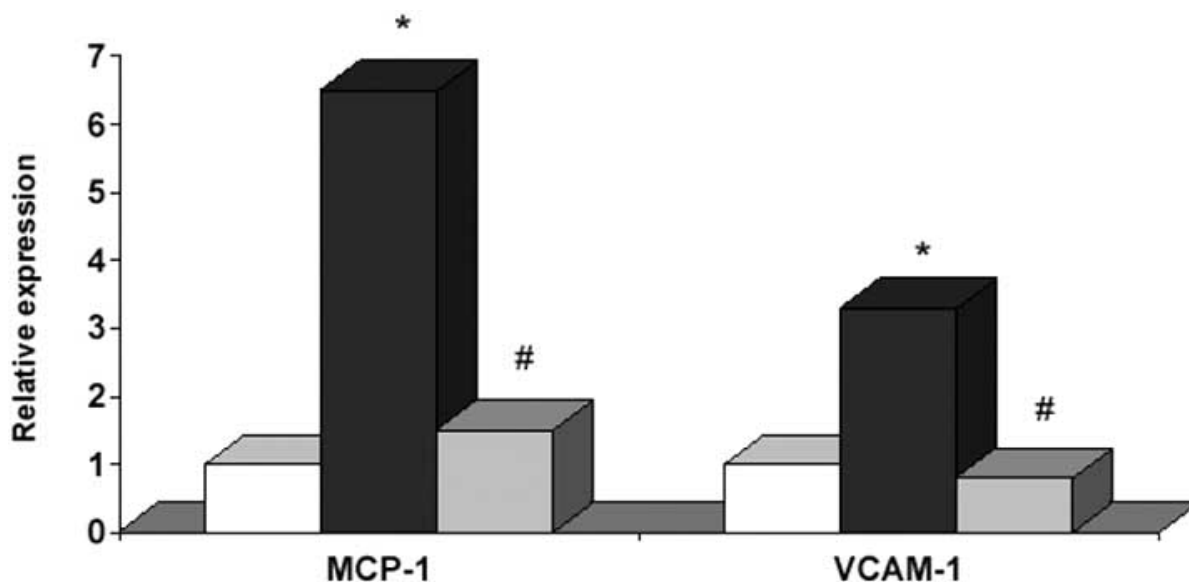
that AGE-receptors have a key role in the development and progression of end-organ damage in diabetes.

Both *in vitro* and *in vivo*, AGE-adducts are able to activate endothelial cells and mononuclear phagocytes. This interaction triggers the activation of secondary messenger pathways such as PKC [58], and increase production of inflammatory and fibrogenic growth factors and cytokines, including TGF- $\beta$  [59], CTGF [60], PDGF [61], TNF- $\alpha$ , IL-1 and IL-6 in the vessel wall [62, 63]. Indeed, in our recent

studies, we have demonstrated the upregulation of CTGF in the plaques from diabetic Apo E knockout mice (Fig. (3)) that appear to be AGE-dependent [46]. Chemokines that are involved in the recruitment of infiltrating cells are also unregulated in response to AGEs, and this phenomenon is also observed *in vivo* (Fig. (4)). For example, MCP-1 is induced by AGE interactions *via* RAGE and NF- $\kappa$ B dependent pathways [64]. Endothelial cells may also react with AGEs to promote cell adhesion, transendothelial migration



**Fig. (3).** Expression of CTGF protein as detected by immunohistochemistry (left) and gene expression by *in situ* hybridization (right) in control (top) and diabetic Apo E knockout mice (Bottom).



**Fig. (4).** Quantification of MCP-1 (left) and VCAM-1 (right) in aortae of diabetic ApoE knockout mice as measured by real time RT-PCR. \* vs control, # vs diabetic  $p < 0.01$ .



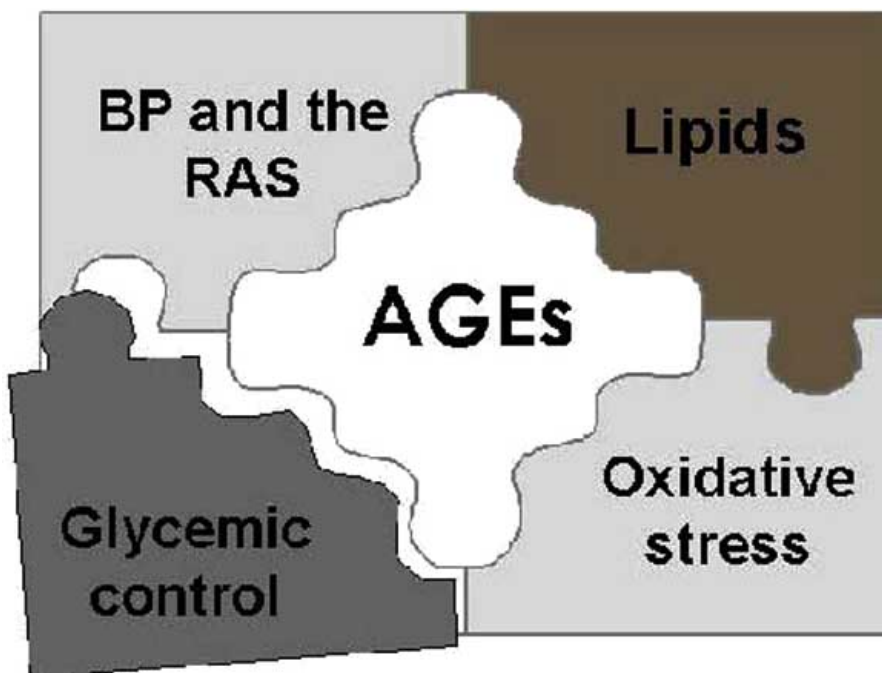
[65], inflammation and the formation of blood clots [66]. Furthermore, smooth muscle cell proliferation is enhanced by AGEs by way of galectin-3 and RAGE activation. Intracellular accumulation of AGEs may also promote phenotypic conversion of smooth muscle cells to foam cells within atherosclerotic plaques [67]. Tethering of AGEs to the cell surface by their receptors also focuses on some of the non-receptor-mediated actions of AGEs onto cellular targets, potentially contributing to increased oxidative stress and the quenching of NO [68]. That each of these effects are predominantly receptor-mediated, is supported by studies using specific antibodies to AGE-receptors to block these changes [69, 70].

Vascular disease in diabetes is associated with the accumulation of matrix proteins within the vessel wall, of which AGEs represent one contributing pathway. While some of these matrix changes are receptor independent (see above), activation of AGE receptors also contributes to vascular fibrogenesis. For example, the expression of extracellular proteins such as fibronectin, type I and type IV collagen is directly increased by AGEs in a dose and time dependent manner, both in the presence [71] or absence of hyperglycemia [72]. This is thought to be predominantly *via* the induction of profibrotic cytokines and growth factors including TGF  $\beta$  1 and CTGF [73, 74]. At the same time that matrix synthesis can be augmented by AGEs, matrix protein degradation and turnover may also be reduced by AGE-modifications. Not only are AGE-modified proteins more resistant to enzymatic digestion, but diabetes is also associated with a reduction in the matrix-degradative capacity [75], through the decreased expression and activity of matrix metalloproteinases and increased activity of metalloproteinase inhibitors through receptor dependent mechanisms [76].

Recently, understanding of the functions of RAGE has been further complicated by the recognition that there are, in fact, three splice variants [77]. These are firstly the full-length RAGE receptor, secondly, the N-terminal variant that does not contain the AGE-binding domain and thirdly, a C-terminal splice variant, soluble RAGE, which contains neither the transmembrane nor effector domains. The function and role of the differential expression of these variants remains to be established. The soluble form of the receptor, sRAGE, has been identified as having potential therapeutic value in experimental atherosclerosis, since injection of this molecule protects against vascular injury in experimental models [78]. It is conceivable that sRAGE acts both as a scavenger for soluble AGEs and competes for binding and activation of cell surface RAGE. Understanding factors affecting the balance between synthesis of soluble RAGE and full-length RAGE will be important for elucidating the mechanism of AGE- and RAGE-induced pathology [79]. For example, the expression of RAGE may be partly under genetic control, and polymorphisms of RAGE may be associated with CVD [80].

#### AGEs AND CARDIAC FUNCTION

Diabetes is commonly associated with heart failure [81]. Between 20 and 40% of all patients with heart failure, have diabetes [82]. In addition, the prognosis of heart failure is significantly worse in patients with diabetes than in non-diabetic patients [83]. While some of this excess relates to macrovascular disease, cardiac fibrosis and hypertrophy may also occur prior to large vessel changes. In the normotensive STZ model, diabetic hearts are characterized morphologically by interstitial changes including an upregulation of fibrillar collagen (Fig. (5)) [84]. The matrix proteins retained in the diabetic heart are also significantly 'stiffer', as



**Fig. (5).** AGEs interact with a variety of pathogenic pathways implicated in the development and progression of end-organ damage in diabetes.

assessed by the reduced proteolytic digestibility of myocardial collagen.

The pathogenesis and mechanisms underlying these early abnormalities in the diabetic heart have not been fully elucidated. However, it is clear that classical risk factors (glycemic control, hypertension, dyslipidemia) only partly explain this increased risk. There is now evidence that AGEs may be considered pivotal mediators of cardiac dysfunction in diabetes. Not only does the AGE-modification of interstitial collagen have a direct effect of cardiac distensibility and contractility, but increased expression and activation of the AGE receptors on cardiac myocytes may also have functional correlates. For example, activation of RAGE directly influences myocardial calcium homeostasis [85] and contributes to interstitial fibrogenesis [84, 86]. Furthermore, inhibition of cardiac AGE accumulation prevents diabetes and age-associated increases in myocardial stiffness, both by decreasing myocardial collagen expression as well as improving collagen digestibility, facilitating the turnover of senescence matrix molecules. These findings are likely to be relevant to the clinical context with recent findings demonstrating that in patients with diabetes, circulating levels of AGEs are correlated with cardiac dysfunction [87].

### THE SYNERGY BETWEEN AGEs AND OTHER PATHOGENIC PATHWAYS

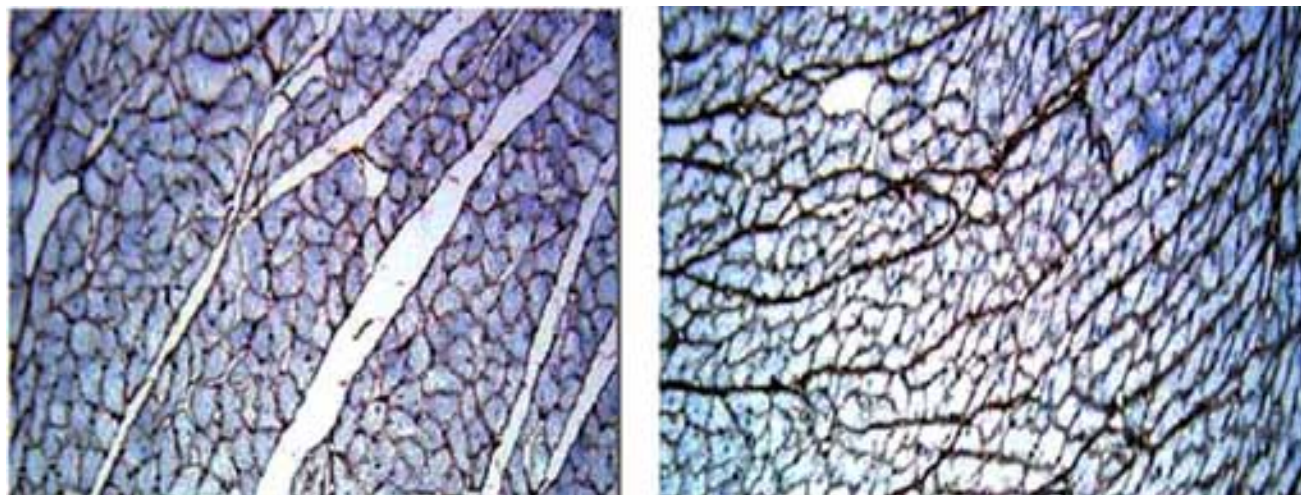
While AGEs have a number of direct effects on the cardiovascular system, it is now clear that AGEs also act in a synergistic manner to potentiate the activity of other pathways implicated in the development of accelerated CVD in diabetes (Fig. (6)).

*Oxidative stress* is a key mediator of pathogenic changes in the diabetic vasculature. It is now apparent that there is a synergistic relationship between AGEs and oxidative stress. Oxidative stress is increased in diabetic animals in proportion to the accumulation of AGEs [88]. AGEs increase the expression and activity of NADPH oxidase, an important source of vascular oxidative stress in diabetes [89]. AGEs can also directly enhance the formation of free radicals through catalytic sites in their molecular structure, possibly

involving the binding and activation of transition metal ions [37]. In addition, AGEs are able to deplete cellular antioxidant systems such as glutathione peroxidase [90]. Glycation of antioxidants such as Cu-Zn-superoxide dismutase also contributes to the decline in antioxidant activity [91]. Important recent studies show that AGEs have also suggested an important role for oxidative stress in RAGE-mediated signaling [92]. AGEs interact with a number of mitochondrial sites, now seen as key targets for diabetes associated end-organ damage, where they are able to induce mitochondrial dysfunction. However, AGE synthesis can be also inhibited by mitochondrial antioxidants [3]. Such synergistic interactions between oxidative stress and advanced glycation pathways in diabetes offer the hope of increased efficacy for combination interventions that target both advanced glycation and oxidative stress ('glycooxidation').

*Dyslipidemia* contributes to accelerated macrovascular disease in patients with diabetes. In addition, advanced lipoxidation end products (ALEs), generated on proteins during lipid peroxidation, accumulate in diabetic renal and vascular lesions [93]. Like AGEs, ALEs have been shown to stimulate inflammatory and fibrogenic cytokine production leading to increased extracellular matrix accumulation and macrophage influx. The relationship between AGEs and dyslipidemia is further complicated by the fact that AGEs also contribute to diabetic dyslipidemia. For example, glycation of the low-density lipoprotein (LDL) receptor and plasma lipoproteins results in impaired cholesterol uptake [50], that may contribute to cholesterol accumulation in diabetic patients.

Like AGEs, ALEs also enhance production of ROS, which autocatalyzes glycooxidation and lipoxidation. The relationship between glycooxidation and lipoxidation is further illustrated by the fact that different of AGE/ALE inhibitors, including aminoguanidine, ALT-711 and pyridoxamine, are able to reduce circulating cholesterol levels [94, 95]. The mechanism of these hypolipidemic actions may be important in patients with diabetes, who require intensive lipid interventions to achieve therapeutic targets. Indeed, in the Heart Protection Study [96], even in type 1 diabetic patients with 'normal' or only modest elevations in lipid



**Fig. (6).** Trichrome stain of myocardial tissue demonstrating interstitial staining for collagen in control (left) and diabetic rats (right).

levels, a reduction in LDL cholesterol levels conferred cardioprotective effects.

**The Intra-Renal Renin-Angiotensin System (RAS).** Activation of the intra-renal renin-angiotensin system has been strongly implicated in the pathogenesis of diabetic vascular complications [97]. Interruption of the RAS with drugs such as angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor antagonists (ARAs) are currently the most effective clinical interventions for both the prevention and treatment of diabetic complications [98]. Beneficial effects, beyond those achieved by blood pressure control alone, have been noted in numerous experimental and clinical trials in diabetes. For example, blockade of the RAS in diabetic apo E KO animals using ACE inhibitors or ARAs results in attenuation of atherosclerosis [6, 99]. AGEs have been shown to significantly interact with the RAS. For example, ACE inhibition significantly attenuates the formation and accumulation of AGEs in experimental diabetes (see below). *In vitro* studies have described the prevention of AGE-associated-induced collagen production by the ACE inhibitor, captopril, suggest that this may occur by reducing RAGE expression and JAK2/STAT activity [69]. In addition, recent evidence in cell culture suggests that AGEs may independently activate the RAS and promote pathways

leading to the formation of Angiotensin II [100]. What is the cause or consequence of vascular injury remains to be established in these models. However, it appears most likely that the RAS and advanced glycation represent complementary and potentially synergistic mechanisms for diabetic vascular injury, leading to a vicious cycle of injury, which may be partly attenuated by inhibition of either pathway [101].

### THE INHIBITION OF AGE ACCUMULATION

A variety of agents have been developed to reduce the accumulation of AGEs in diabetes (Fig. (7)). The fact that the majority have been shown to be effective in experimental models, despite their disparate mechanisms of actions, supports the pathogenic role of AGEs in diabetic end-organ damage. Nonetheless, the clinical utility of AGE inhibition remains to be established.

### AMINO GUANIDINE

*Aminoguanidine* is perhaps the best-known and experimentally, the most widely used inhibitor of advanced glycation. It was the first agent of this class to be shown to attenuate the development and progression of vascular complications in experimental diabetes [102]. However, the

Proposed mechanisms of activity for inhibitors of AGE accumulation

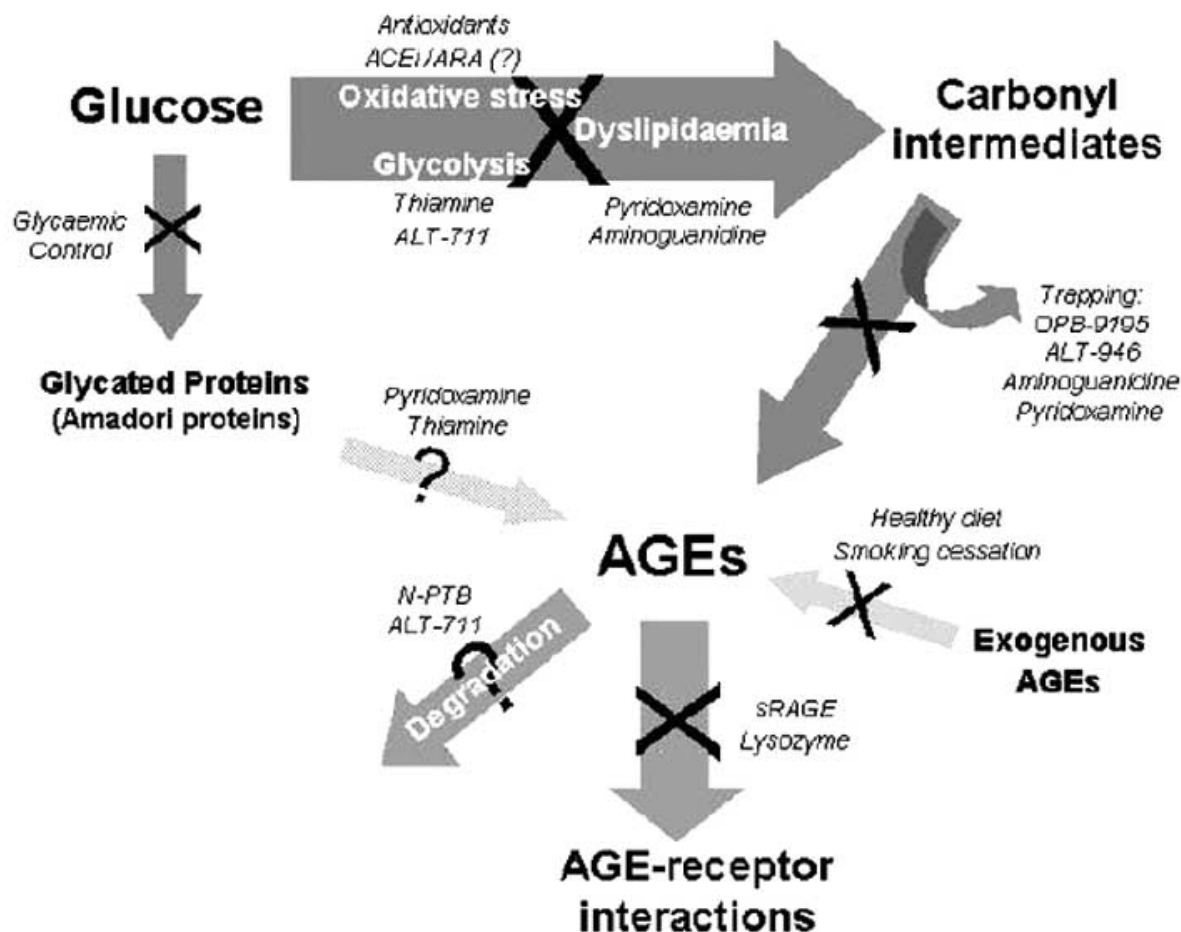


Fig. (7). Proposed mechanisms of activity for inhibitors of AGE accumulation.



complex pattern of metabolic effects seen following treatment with aminoguanidine, over-and-above inhibition of the formation of AGEs, has made the interpretation of these studies problematic, and ultimately raised questions about their applicability in the clinical setting. Aminoguanidine is a hydrazine derivative first described as an agent to prevent formaldehyde toxicity [103]. Subsequently, a number of studies have demonstrated that the cross linking reaction between proteins and glucose-derived dicarbonyl and aldehyde products is effectively inhibited by aminoguanidine [104].

Aminoguanidine is thought to prevent glucose-induced formation of AGEs by its reaction with electrophilic, carbonyl intermediates, thereby protecting against chemical modification of nucleophilic residues on proteins and lipids (Fig. (8)) [102]. For example, following incubation of aminoguanidine with reducing sugars, the concentration of the active aldehyde form of the sugar is decreased, effectively preventing it from modifying amino groups in the protein. However, high molar ratios of aminoguanidine to glucose do not reveal anything about the ability of aminoguanidine to inhibit AGEs *in vivo*, as it will react with any or all open chain sugars in the test tube, until it is used up.

Aminoguanidine is also a potent inhibitor of semicarbazide-sensitive amine oxidase, an enzyme involved in the generation of formaldehyde and methylglyoxal associated with diabetes. Additional protection afforded by aminoguanidine against the formation of AGEs may also be related to direct antioxidant effects [105], including inhibition of catalase activity as well as the scavenging of peroxynitrite radicals [106] and transition metal-chelating activity [107]. Aminoguanidine also increases the activity of S-adenosyl-methionine decarboxylase, leading to augmented production of spermine and spermidine [108]. It is not clear whether this is a direct effect or is mediated by a reduction of nitric oxide, a known inactivator of polyamine synthesis. However, it is important to note that polyamines have been shown to prevent dicarbonyl modification of protein *in vivo*, probably by acting as carbonyl scavengers [109]. Aminoguanidine is also a moderately selective inhibitor of *inducible* nitric oxide synthase (iNOS) [110]. While this action does not contribute

to its ability to reduce AGEs *in vivo* [111], it may explain some of its physiological effects. New, more selective, inhibitors of advanced glycation such as ALT-946 [N-(2-Acetamidoethyl)hydrazinecarboximidamide hydrochloride] have been developed which, unlike aminoguanidine, do not appear to be as potent at inhibiting NO synthase [112]. Unfortunately, none of these compounds have yet proceeded into clinical trials.

There is some evidence from experimental studies, that labeled aminoguanidine can become irreversibly bound to glycated proteins, probably by reacting with protein carbonyl modifications [113]. Although such a reaction would potentially block the formation of a *de novo* crosslink, such as pentosidine, the newly formed adduct would still represent a site of irreversible protein modification. In addition, recent data suggest that aminoguanidine also reacts with pyridoxal phosphate in the liver and kidney, where it forms a Schiff base adduct and a concomitant decrease in the available pyridoxal phosphate (and potentially vitamin B<sub>6</sub> depletion). In contrast, the aminoguanidine pyridoxal-Schiff base adduct appears to have greater efficacy than aminoguanidine, in attenuating diabetic nephropathy and inhibiting glycoxidative modification of proteins in the short term [114], possibly by acting as a slow release form of aminoguanidine.

Several studies in diabetes animals have shown that aminoguanidine is able to reduce both vascular and serum levels of AGE-modified proteins [115]. Moreover, studies in the STZ diabetic rat have shown that aminoguanidine when administered from the induction of diabetes, attenuates the development of vascular complications [116] including nephropathy [4], retinopathy and accelerated atherosclerosis [6]. Further, the greater than three-fold increase in plaque area associated with diabetes in Apo E knockout mice is attenuated following treatment with aminoguanidine [6]. This change was predominantly seen in the thoracic and abdominal aortas, with little change in the aortic arch. These findings are consistent with the dominant role of hemodynamic rather than metabolic factors in the pathogenesis of changes at the arch. Notably, diabetes-associated accumulation of AGEs in the thoracic and abdominal aortas was also ameliorated by aminoguanidine, together with a reduction in

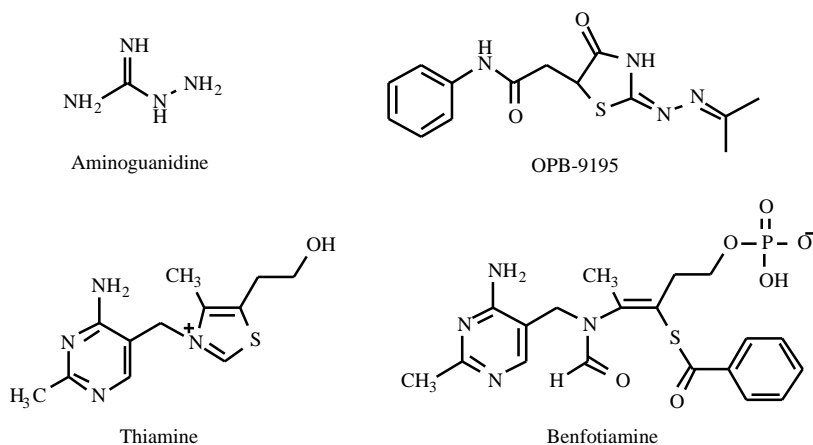


Fig. (8). The chemical structure of aminoguanidine, thiamine, benfotiamine and OPB-9195.

vascular RAGE. Diabetes was also accompanied by aortic accumulation of total collagen, specifically collagens I, III, and IV and increased expression of the profibrotic cytokines TGF- $\beta$  and CTGF. Attenuation of these changes was also seen in diabetic animals treated with aminoguanidine. Similar changes have been shown in other models of accelerated atherosclerosis. For example, aminoguanidine is able to reduce the number of atherosclerotic lesions in animals on a high cholesterol diet [117]. Aminoguanidine is also beneficial in preventing cardiovascular changes associated with age and diabetes, including aortic stiffening and cardiac hypertrophy [118]. However, it should be noted that in many of these studies, high rates of pancreatic and renal-neoplastic tumors have also been reported in rats treated with aminoguanidine [119].

While specific interventions to reduce levels of AGEs have proved highly successful in experimental diabetes, there have only been a few studies attempting to translate these findings into clinical practice. Large clinical studies of *pimagedine* (aminoguanidine) in patients with type 1 diabetes (ACTION 1) and type 2 diabetes (ACTION 2) and overt nephropathy were terminated because of safety concerns. In addition, there was an apparent lack of efficacy in the primary outcome (doubling of serum creatinine). Unfortunately, the protocol was not powered to take into account the beneficial effects arising from concomitant blockade of the renin-angiotensin system in both treatment and placebo groups. Nonetheless, *pimagedine* reduced urinary protein excretion in a sub-population of patients with moderate renal impairment and also inhibited the progression of retinopathy [120]. Smaller studies with aminoguanidine have demonstrated significant improvements in red cell deformability and dyslipidemia in patients with renal disease [121]. However, concerns regarding long-term toxicity of this agent have largely stalled the development of aminoguanidine as a clinically useful inhibitor against the formation of AGEs.

## THIAMINE AND ITS DERIVATIVES

Thiamine (*vitamin B<sub>1</sub>*) is involved in the  $\alpha$ -carbonyl reactions of carbohydrate metabolism, as well as a number of other physiological processes including the synthesis of neurotransmitters, nucleic acids, fatty acids and steroids. High concentrations of thiamine are found in skeletal muscles and in the heart, liver, kidney and brain [122]. However, the discovery of thiamine and its derivatives as potential inhibitors against the formation of AGEs is comparatively recent. In searching for potential inhibitors of late glycation reactions, Hudson and colleagues demonstrated that 3-50 mM concentrations of both thiamine pyrophosphate and thiamine were able to inhibit formation of AGEs in bovine serum albumin co-incubated with 1.0 M glucose [123]. Notably, these *agents* were found to be as effective or more effective than the AGE inhibitor, aminoguanidine, in inhibition of formation of AGEs from Amadori compounds on glycated proteins.

The mechanisms by which thiamine and its derivatives might inhibit the formation of AGEs *in vitro* are poorly understood. The fact that both these *B<sub>1</sub>* vitamers successfully inhibited the formation of immunogenic AGEs produced

from post-Amadori intermediates, in contrast to aminoguanidine, suggests that one their actions *in vitro* is *via* interactions with post-Amadori precursors of AGEs [124]. In addition, thiamine may react with the open chain aldehyde forms of the reducing sugars or with carbohydrate adducts on protein. The stronger inhibition of thiamine pyrophosphate suggests that the negative charge of the amine group is an important component of the reaction. How this may occur has not been defined. Thiamine has a heterocyclic thiazolium ring, similar to that of phenylthiazolium bromide, an agent purported to chemically break AGE cross links (Fig (8)). Some animal studies have found that high doses of thiamine are able to inhibit lipid peroxidation resulting from orally administered lead, which suggests that thiamine may also act as an antioxidant or metal chelator [125].

The protective effect of thiamine *in vivo* may also be related to its physiological functions as a co-enzyme in mitochondrial carbohydrate metabolism. In particular, thiamine in its diphosphate form is an important co-enzyme for transketolase, pyruvate dehydrogenase,  $\alpha$ -oxoglutarate dehydrogenase and the branched-chain  $\alpha$ -oxoacid dehydrogenase complex, enzymes that are involved in energy production pathways [126]. It has been proposed that at least some of the damage induced by high glucose concentrations may be produced *via* direct stimulation of the glycolytic pathway. The increased flux of glucose through glycolysis leads to increased formation of triose-phosphate intermediates, including glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, followed by spontaneous  $\beta$ -elimination of the phosphate and subsequent generation of reactive  $\alpha$ -oxaldehydes [3]. These compounds are several orders of magnitude more active than glucose in generating AGE-modifications [127]. In addition, these glycolytic intermediates are involved in activation of the hexosamine pathway, diacylglycerol (DAG)-protein kinase C (PKC) pathway and NF- $\kappa$ B activation, which are all implicated in the pathogenesis of diabetic vascular disease [128]. As a strategy to reduce triose-phosphate generation in hyperglycemia, supplemental thiamine is thought to increase transketolase activity, thereby increasing glyceraldehyde-3-phosphate metabolism and directing these metabolites toward the pentose-phosphate shunt. This results in the formation of sedoheptulose-7-phosphate and ribose-5-phosphate and the re-direction of pyruvate toward the Krebs cycle [129].

Thiamine also plays an important role in the regulation of glucose metabolism. Indeed, an association between thiamine deficiency and glucose intolerance has been recognized for over 80 years [130]. In thiamine deficient rats, reduced glucose oxidation and insulin secretion is restored by thiamine administration [131]. Thiamine may also be involved in cellular glucose transport [132]. In children with long-standing insulin-dependent diabetes mellitus, metabolic control can also be improved by thiamine supplementation [133]. It has been suggested that part of the association between fibre intake and glucose tolerance may be attributable to concomitant thiamine intake [134]. Of interest, Rogers syndrome, a rare genetic disorder that produces a generalized disturbance of thiamine uptake, also leads to diabetes [135]. Supplemental thiamine may also have distinct effects on lipid metabolism [136] and improve exercise tolerance. Each of these effects may serve to reduce the

accumulation of AGEs and ALEs in individuals with diabetes.

Diabetes *per se* is also associated with functional or absolute thiamine deficiency. This deficiency has been demonstrated in mice rendered diabetic with either STZ or alloxan. A significant proportion of diabetic patients also appear to have mild thiamine deficiency [137, 133, 137-139], associated with a low blood thiamine level, with low erythrocyte transketolase activity [139]. However, some other studies contradict these findings [141, 142]. Thiamine deficiency has the potential to contribute to AGE accumulation *via* a number of different mechanisms. Not only is thiamine deficiency associated with increased glucose intolerance (see above), but metabolism of dicarbonyl intermediates such as glyoxal is inhibited in thiamine-deficient cells [143].

The mechanisms that lead to vitamin B<sub>1</sub> deficiency in diabetes remain to be established. Increased thiamine requirements due to chronic hyperglycemia may provide a partial explanation. Increased renal clearance of thiamine and impaired tubular re-absorption of filtered thiamine in patients with diabetes have also been proposed. Certainly, increased urinary thiamine loss by diuretics is caused by a non-specific, flow-dependent mechanism common to all diuretic agents [144]. However, it is in patients with reduced renal function that we find the highest levels of AGEs and the lowest levels among those with hyperfiltration [145]. Moreover, patients with chronic kidney disease (but not on dialysis) in whom clearance of water-soluble vitamins is reduced also have reduced thiamine levels. Chronic alcohol consumption results in thiamine deficiency due to inadequate nutritional thiamine intake, decreased absorption of thiamine from the gastrointestinal tract, and impaired thiamine utilization [146]. While malnutrition is not a problem in diabetes, insulin deficiency reduces the net transport of thiamine and thiamine monophosphate cations in the intestine [147], and mitochondrial mechanisms implicated in intracellular thiamine transport [148] may also be dysfunctional. Reduced erythrocyte transketolase activity in diabetes may also result from the disease itself, exacerbating any thiamine deficiency [149].

*Beri beri* (a disease resulting from thiamine deficiency) produces a broad spectrum of clinical manifestations including cerebellar degeneration (Wernicke-Korsakoff syndrome), peripheral neuropathy and cardiovascular beriberi (so called 'wet' beriberi). The unique predisposition of these sites is thought to relate to the dependency on glucose as the primary substrate for metabolism [150]. To support this hypothesis, it is well-established that rats do not develop cardiomyopathy in response to thiamine deficiency [151]. However, in animals deficient in macrophage AGE receptor, CD36, there is a shift in the myocardial energy substrate from long chain fatty acids to glucose [152] that renders these animals susceptible to thiamine deficiency [150]. A similar shift in myocardial substrate has also been demonstrated in the hypertrophied and failing heart [153].

Given the association between diabetes and thiamine deficiency, and the key function of this co-enzyme in carbohydrate metabolism, there appears to be a clear rationale for its use in the treatment of diabetes, independent of its ability to inhibit AGEs. However, thiamine itself is poorly absorbed

and taken up into cells. For example, high dose supplementation does not increase intracellular thiamine levels beyond the first 12 mg [154], with the rest being lost into the urine. To overcome this limitation, over fifty years ago, researchers noted that some thiamine-derived compounds found in crushed roasted garlic (so called '*allithiamines*') had improved intestinal and cellular absorption properties. The best studied of the allithiamines is benfotiamine (Fig. (8)), a lipid-soluble thiamine derivative that (depending on the cell type) results in a 5-120 fold increase in intracellular thiamine levels, when compared to an equivalent amount of thiamine. A number of experimental studies have examined the effect of benfotiamine, confirming its potential utility in preventing the microvascular complications associated with chronic hyperglycemia [126, 155]. For example, Hammes and colleagues demonstrated that benfotiamine significantly attenuated retinal damage in diabetic animals [128]. Thornalley and colleagues have also demonstrated its effectiveness in the prevention of diabetic nephropathy [156]. Notably, both studies were able to demonstrate a reduction in the serum levels and tissue accumulation of AGEs, suggesting that this is one of the mechanisms of actions of the drug. The results of proposed clinical studies are eagerly awaited. However, it should be noted that benfotiamine has already been used for many years in the former Soviet Union for the treatment of diabetic neuropathy under the proprietary name of '*Milgamma*'.

The potential utility of thiamine derivatives for the management of macrovascular complications in diabetes remains to be determined. However, thiamine has been found to inhibit human arterial muscle cell proliferation induced by high glucose and insulin in cell culture [157], suggesting that optimal thiamine levels may be useful in delaying the atherosclerotic complications of diabetes. Thiamine and benfotiamine also have effects on apoptosis in endothelial cells subjected to high glucose [158]. Increased mitochondrial free radical production and subsequent inactivation of glyceraldehyde phosphate dehydrogenase in vascular endothelial cells has also been associated with endothelial dysfunction in experimental models [159]. Finally, thiamine deficiency is also associated with a number of cognitive and emotional changes including depression, lethargy and inanition, each known to be risk factors for CVD.

## PYRIDOXAMINE AND OTHER VITAMIN B<sub>6</sub> DERIVATIVES

Vitamin B<sub>6</sub> is known to have an important role in carbohydrate metabolism. The administration of a single dose of a pyridoxamine (or other vitamin B<sub>6</sub> derivatives) results in mobilization of liver glycogen and a concomitant elevation of the serum glucose, since pyridoxal phosphate is an essential co-factor in hepatic aminotransferase activity [160]. Livers from diabetic animals also contain increased concentrations of pyridoxine and pyridoxamine, associated with increased aminotransferase activity and gluconeogenesis due to insulin deficiency [161]. This close association between vitamin B<sub>6</sub> derivatives and glucose metabolism has recently led researchers to investigate their possible interaction with the Maillard reaction.

In the initial studies with pyridoxamine, Hudson *et al.* [124] evaluated the inhibitory activity of analogs of vitamin B<sub>6</sub> in an experimental system in which Amadori-proteins were prepared by short-term pre-incubation of the protein with ribose or glucose under antioxidative conditions that limited the formation of AGEs. Unlike aminoguanidine that was only able to inhibit sugar-derived formation of AGEs, pyridoxamine was also able to inhibit the formation from pre-glycated (Amadori-modified) proteins *in vitro* [162]. The exact mechanism of this activity remains to be fully established. Pyridoxamine adducts of lipoxidation intermediates can be detected both *in vitro* and in the urine of pyridoxamine-treated animals, suggesting that pyridoxamine acts (at least partially) by irreversibly binding reactive intermediates in ALE formation. For example, both N-hexanoyl-PM and N-nonanedioyl-pyridoxamine monoamide, formed in reactions of linoleate with pyridoxamine *in vitro*, can be detected in the urine of pyridoxamine-treated diabetic and obese rats [163]. Other urinary products that have now been identified include the pentanedioic acid amide derivative of pyridoxamine, derived from the  $\alpha$ -terminus of arachidonate and N-formyl-pyridoxamine. Although carboxymethyl-adducts have been described in model *in vitro* reactions [164], carboxymethyl, carboxyethyl, malondialdehyde or 4-hydroxynonenal derivatives of pyridoxamine were not detected *in vivo*, suggesting that pyridoxamine reacts primarily with early intermediates in the formation of AGEs and ALEs. In addition, pyridoxamine appears to be significantly less reactive with aldehydes and dicarbonyl compounds than aminoguanidine, although both readily form Schiff base with aldehydes [165, 166]. Taken together, these findings suggest that the "trapping" activity of pyridoxamine is selective to lipid precursors of carbonyl reactive intermediates such as methylglyoxal. Although similar intermediates derived from intermediates in glycoxidation reactions have not been isolated from plasma or urine, pyridoxamine may also react with sugar-derived ketoaldehydes intermediates such as glucosone, 1-deoxyglucosone and 3-deoxyglucosone. The proposed mechanism of reaction of pyridoxamine with ketoaldehydes suggests that pyridoxamine could also cleave some  $\alpha,\beta$ -dicarbonyl compounds. Indeed, pyridoxamine is able to cleave phenylpropanedione, a proposed model for AGEs cross links in protein [167], suggesting that it too might function like a 'cross link breaker' of AGEs. However, none of the known cross links, eg pentosidine, MOLD and GOLD contain this putative dicarbonyl moiety. In addition, activity in reversing pre-existing cross links in tissue proteins has not been confirmed with pyridoxamine [168].

In addition to its reactivity with AGE/ALE precursors, pyridoxamine may also function as an antioxidant. For example, Onorato *et al.* [169] demonstrated that pyridoxamine inhibits the formation of CML and malondialdehyde-lysine during autooxidation of arachidonate in the presence of RNase and during metal-catalyzed oxidation of LDL. Both pyridoxamine and pyridoxine are also able to reduce superoxide generation and protect red cells against oxidative stress during exposure to high glucose concentration in cell culture [170]. Both compounds also provide modest protection against membrane lipid peroxidation, measured as MDA, and inactivation of Na<sup>+</sup>, K<sup>+</sup>-ATPase by high glucose.

Kinae and colleagues [171] observed that pyridoxamine protects against oxidative damage to DNA during growth of human umbilical vein endothelial cells (HUVECs) in high glucose medium. In addition, pyridoxamine and pyridoxine is able to form stable complexes with transition metal ions including Cu<sup>2+</sup> and Fe<sup>3+</sup>. Despite these data, it is unlikely that pyridoxamine is functioning primarily as an antioxidant or metal chelator in the HUVEC system, since pyridoxamine does not inhibit the glucose-induced increase in generation of intracellular peroxides [172] and does not prevent copper-catalyzed oxidation of fatty acids or LDL. In addition, it is unlikely that pyridoxamine is acting primarily as a vitamin, since all of its effects *in vivo* can be mimicked by aminoguanidine. Moreover, unlike pyridoxamine, B-vitamin supplements do not protect against diabetes-associated vascular damage in diabetes.

Pyridoxamine has also been shown to have direct lipid lowering effects *in vivo*. For example, in both diabetic and non-diabetic animals (*Zucker obese rats*), pyridoxamine is able to reduce plasma triglycerides and cholesterol. The mechanism of this effect remains to be established. However, lipid lowering appears to be independent of prevention of proteinuric renal disease in these models. It has been suggested that these lipid lowering effects may significantly contribute to the reduction in AGEs seen with pyridoxamine *in vivo*. For example, dyslipidemia leads to the formation of ALEs such as CML and CEL [173], which are reduced following pyridoxamine. By contrast, AGEs that are not derived from lipids, such as pentosidine, are unaffected by either pyridoxamine or aminoguanidine. Furthermore, hypolipidemic therapy with statins or fibrates have both been shown to be protect against vascular damage in experimental diabetes [174]. Consequently, it may be unnecessary to invoke direct AGE inhibitory activity to explain the beneficial effects of the drugs.

Regardless of its primary mechanism of action, experimental studies have confirmed that pyridoxamine is a potent inhibitor of AGE accumulation in diabetes. For example, pyridoxamine prevented the increase in CML, CEL, Maillard-type fluorescence and cross-linking of skin collagen in diabetic rats, with an effect comparable to that of aminoguanidine. More recently, Nagaraj *et al.* [175] reported that pyridoxamine inhibited modest increases in MOLD (and pentosidine concentration in plasma proteins), and also inhibited a nearly 3-fold increase in plasma and erythrocyte methylglyoxal concentration. Degenhardt *et al.* have shown that pyridoxamine is able to attenuate the development of renal disease, as measured by increases in urinary albumin and glomerular volume and plasma creatinine in the STZ-induced diabetic rat [176] as well as in the db/db mouse [177]. Similarly, pyridoxamine has recently been shown to retard the development of retinopathy [178] and neuropathy in the STZ-diabetic rat [179]. In the Zucker rat, pyridoxamine also protects against renal disease, inhibiting the development of both albuminuria and renal impairment. In addition, pyridoxamine also prevents the increase in systolic blood pressure and mean arterial pressure in the Zucker rat, as well as inhibiting thickening of the aortic wall and the walls of small arteries in the heart and kidney. A direct vasculoprotective effect of pyridoxamine is further supported

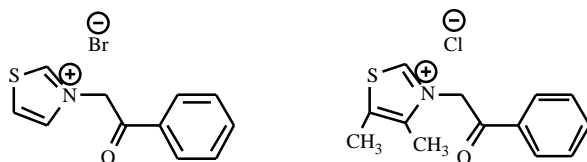


by preliminary studies in diabetic Apo E knockout mice [180].

In Phase II clinical trials, pyridoxamine has proven to be safe and well-tolerated. Although not designed to test this endpoint, *post hoc* analyses of these studies noted significant reductions in the rise of serum creatinine and urinary albumin excretion in the treatment group [181]. In another human study, a reduction in the urinary excretion of the pro-fibrotic cytokine TGF $\beta$  was also demonstrated. Future phase III studies in patients with diabetes, particularly in patients with atherosclerotic vascular disease, are eagerly awaited.

### THE "CROSS LINK BREAKERS", PTB AND ALAGEBRIUM CHLORIDE

Another group of compounds that may prove useful in reducing levels of AGEs are the so-called 'cross link breakers' [N-phenacylthiazolium bromide (PTB) and alagebrium chloride (ALT-711)] (Fig. 9) [182]. However, the designation of these compounds as 'AGE-breakers' may yet prove to be a misnomer. Although these compounds are clearly able to release AGE-modified proteins from highly modified *ex vivo* samples, it remains to be firmly established whether these agents are actually able to break established cross links of AGEs *in vivo* [168]. Nonetheless, it is clear from both clinical and experimental studies that these agents, originally identified for their putative activity in breaking AGEs, are potent inhibitors of tissue AGE accumulation in diabetes and have beneficial effects on phenomena associated with AGE accumulation, including the aging of the cardiovascular system.



**Fig. (9).** The structure of cross link breakers, N-PTB (left) and alagebrium chloride (right).

It has been known for some time that the carbon-carbon bond of alpha-diketones can be selectively cleaved with certain thiazolium salts [183]. For example, in the test tube, N-PTB rapidly cleaves the model diketone, phenylpropanedione, with release of benzoic acid [184]. Working on the assumption that an intermediate containing two adjacent carbonyl groups may be important in the formation of intermolecular cross links, PTB was initially synthesized as an agent to selectively cleave the diketone bridges of AGEs [167]. And indeed, when PTB was incubated with tail collagen from diabetic animals, it restored the fragmentation pattern on cyanogen bromide digestion to that seen in healthy control animals, suggesting that it was able to disrupt diabetes associated (AGE-mediated) cross linking [182]. However, other attempt to reproduce this data have been unsuccessful [186]. PTB has also proved useful for releasing DNA trapped within matrices of sugar-derived condensation products, making it possible to amplify DNA sequences from ancient samples. For example, PTB has been a key factor in

the extraction of DNA from pre-historic remains, specifically Neanderthals, Mammoths and Giant Ground Sloths [185]. PTB is a valuable tool for forensic and ancient DNA laboratories that routinely deal with the extraction of DNA from degraded tissue.

Initial studies with PTB also demonstrated that treatment of diabetic rat erythrocytes *ex vivo* with PTB also resulted in the release of covalently bound IgG from the red cell surface by AGE-crosslinks. Crucially, this result was able to be replicated *in vivo*, such that treatment of diabetic animals (10 mg/kg four times per day for up to 4 weeks) resulting in a 60–70% reduction in cell surface IgG. This series of experiments supported the hypothesis that the biological activity of PTB may be linked to the specific cleavage of the alpha-dicarbonyl-based protein cross links. However, there is currently little evidence for  $\alpha,\beta$ -dicarbonyl compounds *in vivo*. Moreover, under physiological conditions, PTB undergoes rapid hydrolysis and cyclic hemithioacetal formation [186]. Consequently, it is likely that that other actions of this compound contribute to its efficacy *in vivo*. To this end, it is possible that PTB interacts with methylglyoxal and other dicarbonyl precursors of AGEs, rather than the AGEs themselves [187]. In addition, there is now good evidence that PTB and other thiazolium salts are strong chelators of transition metals, and thus inhibit both enzymatic and non-enzymatic crosslinking of collagen, i.e., by inhibiting metalloenzymes or metal-catalyzed glycooxidation reactions. There is also evidence that thiazolium compounds may independently have antioxidant activity [188] and inhibit auto-oxidation of ascorbate [107] and glucose [188], and thus inhibit formation of AGEs. PTB is also able to promote the metabolism of triosephosphates, possibly by indirectly stimulating transketolase activity through the physiological co-factor, thiamine pyrophosphate [156]. Indeed, many of the thiazolium derivatives have an active site similar in structure to the catalytic ring of thiamine.

Regardless of their mechanism of activity, there is good evidence that thiazolium compounds are able to reduce vascular AGEs in experimental diabetes. For example, in one study, we treated STZ diabetic rats with PTB for three weeks [189]. Notably, accumulation of AGEs in the mesenteric vasculature associated with diabetes was prevented. More recently, we have shown that a more stable thiazolium compound, alagebrium chloride [4, 5-Dimethyl-3-(2-oxo-2-phenylethyl)-thiazolium chloride] is able to reduce accumulation of AGEs when given as a delayed intervention [190]. These results were confirmed by Schwedler who observed that PTB caused a decrease in renal AGEs and increase excretion of AGEs in urine [191]. Unexpectedly, Oturai *et al.* failed to detect any effect of PTB administered from time of onset of diabetes, on the decrease in tail collagen solubility in STZ-diabetic rats [192].

A number of studies have also confirmed the utility of thiazolium compounds in the prevention and reversal of vascular complications of diabetes. We have previously demonstrated that ALT-711 is able to attenuate the progression of microvascular renal disease in animals with diabetes, associated with a reduction in tissue and plasma levels of AGEs [24]. More recently, we have shown that alagebrium is able to attenuate the development of atherosclerosis in

diabetic ApoE deficient mice, an established model of atherogenesis [6]. Treatment of animals with STZ-induced diabetes with PTB for 1-3 weeks also reverses diabetes-induced increases in large artery stiffness as measured by systemic arterial compliance, aortic impedance, and carotid artery compliance and distensibility [193]. Similar beneficial effects have been seen in small vessels in diabetes (e.g. in the mesenteric bed) [10].

The beneficial effects of PTB and alagebrium are also seen in non-diabetic models of disease associated with AGEs. In particular, alagebrium has been shown to prevent increases in vascular and myocardial stiffness in aged animals [194, 195]. Spontaneously hypertensive rats also show a reversal of aortic stiffening, along with a restoration of left ventricular elasticity after treatment with alagebrium [196]. Finally, of cosmetic interest, topical application of alagebrium improves the water content and elasticity of the aged rat skin, compared to that of control animals [182]. Taken together, the results suggest that acylthiazolium compounds show considerable potential for reduction of vascular complications of diabetes and aging, although not necessarily achieved by cleavage of AGEs. Certainly, alagebrium is associated with improvements in arterial pulse pressure [197] and dyslipidemia [213], both of which may independently contribute to intravascular oxidative stress and atherogenesis.

ALT-711 also has important effects on cardiac function, consistent with the key role of protein cross linking in ventricular stiffness in experimental diabetes. For example, ALT-711 is able to attenuate diabetes associated cardiac fibrosis and improve cardiac function. These effects occurred in the setting of improved cardiac collagen solubility, reduced accumulation of AGEs, a decrease in protein expression of RAGE, less CTGF and type III collagen expression and an improvement in cardiac function with attenuation of cardiac BNP expression [86]. These findings complement those seen in aging animals, where age-related cardiac pathology may be attenuated by ALT-711 [196, 198]. Whether these effects are direct or relate to positive effects on vascular tone in aging and chronic disease remains to be established.

To date, there have been no clinical studies of alagebrium in patients with diabetes. However, clinical studies in other conditions suggest that the 'crosslink breakers' may be of some value in reducing the cardiovascular burden associated with diabetes. In *phase 2a* double-blind trial, 62 patients with isolated systolic hypertension took a dosage of 210 mg per day for 8 weeks, while 31 other individuals received a placebo dose [198]. Patients receiving alagebrium experienced statistically significant improvement in large artery stiffness and reduced arterial pulse pressure [198]. Preliminary results from the DIAMOND (Distensibility Improvement and Remodelling in Diastolic Heart Failure) study also demonstrated reductions in left ventricular mass and improvement in left ventricular diastolic filling following treatment with alagebrium. This was manifested clinically by improvements in their New York Heart Association (NYHA) class and quality-of-life measurements (data presented at The Society of Geriatric Cardiology, 2003) [199]. Future studies of this agent in patients with vascular complications of diabetes are keenly awaited.

## OPB-9195

*OPB-9195* [(+/-)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide] belongs to a group of thiazolidine derivatives, which are used predominantly as hypoglycemic agents. Although OPB-9195 itself does not lower blood glucose levels, it inhibits the formation of AGEs in a dose-dependent fashion. This effect is observed with a number of different substrates *in vitro* including glucose, ribose and ascorbate, and includes effects on the formation of different AGEs including pentosidine and CML [200]. Moreover, OPB-9195 appears to be more effective than aminoguanidine in inhibiting *in vitro* pentosidine formation in uremic plasma [201]. In addition, OPB-9195 has been shown to reduce serum concentrations and tissue deposition of AGEs in experimental diabetes [202].

The mechanism of action of OPB-9195 remains to be fully established. At least some of its *in vitro* activity may relate to the trapping of reactive carbonyl intermediates as well as repression of their production [203], thereby inhibiting the formation of carbonyl-based AGEs such as pentosidine and CML. For example, treatment of murine thymocytes and fibroblasts with glyoxal results in tyrosine phosphorylation of multiple proteins, an effect which was inhibited by OPB-9195. It has been suggested, in a mechanism similar to that proposed for aminoguanidine, that the hydrazine nitrogen atom of OPB-9195 is able to react with carbonyl groups, generating hydrazone derivatives [204].

The hypothesis that thiazolidine agents are predominantly dicarbonyl scavengers is supported by the observation that OPB-9195 is able to reduce the levels of carbonyl intermediates present in heat-sterilized peritoneal dialysis fluids in the absence of protein. OPB-9195 also inhibits formation of ALEs in the presence of arachidonate, possibly by a similar mechanism. It is possible that some of this activity may be mediated by its ability to chelate transition metals, and thereby prevent the auto-oxidation of sugars and reduce overall metal-catalyzed oxidative damage to proteins *in vivo* [107]. In addition, metal chelation may remove weakly-bound transition metal ions from these AGEs, and thereby preventing metal-catalyzed oxidative damage.

In studies in experimental animals, OPB-9195 was a potent inhibitor of microvascular injury including the development of diabetic nephropathy. In one study in Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats, a Type II (non-insulin-dependent) diabetes model, treatment with OPB-9195 slowed the progression of mesangial expansion and glomerulosclerosis, as well as reducing deposition of AGEs in diabetic glomeruli [205]. In animals over expressing RAGE, in whom microvascular damage is exacerbated in the setting of diabetes, OPB-9195 was also beneficial in attenuating nephropathy [206]. Notably, oral administration of OPB-9195 suppressed the development of the renal insufficiency, nephromegaly and glomerulosclerosis in RAGE over expressing animals, without affecting the levels of blood glucose or HbA<sub>1c</sub>. Similar beneficial effects have been observed in neuropathy in STZ diabetic rats [207]. Recently, effects outside the setting of diabetes have also been observed. For example, in rats (following balloon injury of their carotid arteries), administration of OPB-9195

was able to effectively inhibit the development of intimal thickening and vascular occlusion [208]. In addition, OPB-9195 is able to reduce blood pressure levels and prevent vascular accumulation of AGEs in stroke-prone spontaneously hypertensive rats [209], a model of essential hypertension.

Despite its beneficial effects, OPB-9195 is no longer being evaluated for clinical applications. Because of its reactivity with carbonyl compounds, it interferes with metabolism of vitamin B<sub>6</sub> (pyridoxine) and contributes to vitamin B<sub>6</sub> depletion by forming a Schiff-base adduct. The merits of administering this compound as a complex with Vitamin B<sub>6</sub> deserves serious study.

### SOLUBLE LIGANDS FOR AGEs

Another approach for decreasing levels of circulating AGEs has been to provide soluble receptors to compete with cellular receptors for AGE-binding, and thereby reduce endogenous activation and resulting oxidative stress in the vessel wall and, in turn, further AGE generation. binding of circulating AGEs and low molecular weight receptors may facilitate the removal of circulating AGEs *via* renal filtration.

As outlined previously, there are three splice variants of RAGE [210], including a C-terminal splice variant, soluble RAGE, which does not contain the transmembrane and effector domains but retains its ability to bind AGEs. Soluble RAGE (sRAGE) is found in serum and mucosal secretions, where it is thought to be involved in targeting senescent molecules for degradation. There have now been several studies, which have shown that sRAGE is able to modify AGE-mediated activation of pathways implicated in the development of diabetic nephropathy [211] and macrovascular injury [212]. In particular, late administration of sRAGE is also able to reduce atherosclerosis when used as a late intervention in diabetic apo E mice [212], and prevent restenosis in a balloon injury model of vascular damage [213]. Although these findings confirm a role for RAGE activation in accelerated atherosclerosis in murine models, the clinical utility of sRAGE remains uncertain. Nonetheless, blockade of the receptor represents an enticing target for future therapies designed to reduce oxidative stress and AGE-mediated vascular injury in diabetes.

Lysozyme and lactoferrin are naturally occurring antibacterial defense proteins found in mucosal secretions, serum, and in the lysosomes of phagocytes. Both molecules contain conserved 18 amino-acid cysteine-bounded domains with high affinity binding for AGE-modified proteins and lipids ( $K_d$  50 nM) [214]. The potential importance of this interaction is illustrated by the fact that Sepharose 4B conjugated with lysozyme is able to deplete AGEs from the sera of diabetic patients with end stage renal disease, without significant protein loss (< 3%) [49]. The precise epitope responsible for the binding of AGE-modified proteins to lysozyme remain to be identified, however, it appears that modifications other than CML and pentosidine may not be directly involved.

In diabetic mice, the binding of AGEs to lysozyme is thought to act as an opsonizing factor, enhancing the uptake and degradation of AGE-modified proteins by macrophages

*via* the AGE-receptor pathway [215]. In both the non-obese diabetic (NOD) and db/db (+/+) mice, an intraperitoneal injection of lysozyme significantly reduced the serum AGE levels and enhanced urinary excretion of AGEs detected by an anti-CML antibody. Moreover, lysozyme also reduced albuminuria in these models, associated with improved macrophage and mesangial cell function [216]. Although the utility of this pathway in a clinical setting remains to be established, a number of interventions are known to increase serum lysozyme activity, including caffeine intake.

### EXPOSURE TO EXOGENOUS AGEs

Food chemists have known for some time that AGEs can be formed during fermentation, during prolonged or high temperature heating or cooking. Consequently, certain processed foods have higher levels of AGEs than others. For example, foodstuffs that have been browned or caramelized (e.g. *colas*, *coffee*, *soy sauce*, *cooked duck skin*) tend to have the highest levels of AGEs [217]. Browning and glycoxidation may also occur during long-term storage, causing foodstuffs to become stiffer and less digestible. Indeed, AGE-modifications of stored food can serve as parameters of food quality [218]. Finally, the formation of AGEs may also be a normal part of intestinal digestion.

The modification of protein by AGEs has a number of dietary consequences, both positive and negative. Many of the 'browning' effects involved in cooking produce flavors or aromas that give many foods their appeal. In addition, incorporating synthetic AGEs into foods can boost the flavor of natural foods. It has been suggested that this fact alone has led to the AGEs content of Western diet becoming significantly increased over the last 50 years, over and above the increased quantity of food consumed. However, the modification of protein by AGEs also reduces nutritional value, as AGEs-modified amino acids cannot be incorporated into new protein during protein synthesis or utilized *via* other metabolic pathways. Furthermore, there is accumulating evidence of a correlation between circulating levels of AGEs and the level of AGEs consumed. For example, patients fed a diet that included AGE-modified egg white showed elevations in serum levels of AGEs proportional to the amount ingested [219]. In addition, the urinary excretion of AGEs appears to be strongly associated with their dietary intake [220]. The exact nature of this association is controversial. Since intact AGE-modified proteins do not survive gastrointestinal proteolysis, ingested AGEs can be absorbed only as low-molecular weight AGEs, which are transported across the intestinal wall, enter the circulation and are subsequently cleared by the kidney. Consequently, levels of AGEs may increase as a result of increased intake or decreased clearance, such as in patients with renal impairment or cirrhosis. Regardless of renal function, however, the content of AGEs in LDL of diabetic patients appears to increase following an AGE-rich diet and to correlate strongly with the AGE content of the diet. Nonetheless, hyperfiltration may initially protect patients with diabetes from accumulation of AGEs due to increased renal clearance of low molecular weight compounds. However, as renal impairment progresses, there is a steady increase in plasma AGEs; in fact, plasma AGEs correlate well with plasma creatinine concentration.

It is not necessary that intact AGEs be absorbed from the diet to affect an increase in plasma AGEs. AGE-precursors, such as methylglyoxal and other reactive carbonyl compounds, may be taken up from the diet, forming AGEs in plasma. The levels of these precursors may correspond to the level of AGEs in the diet, so that plasma AGEs increase in response to the AGE content of the diet. For example, LDL from diabetic patients exposed to a diet with a high glyco-oxidant content is more extensively modified than LDL from subjects fed a diet containing reduced AGEs, reflecting differences in CML and MGO derivatives. It is also conceivable that the increase in protein-modification associated with a high glycooxidant diet are predominantly ALEs, derived from ingested lipid peroxides. Following meals rich in oxidized lipids, these ALE-precursors may be transported into plasma as chylomicrons and are subsequently incorporated into other lipoprotein particles including LDL. Thus, the apparent increase in AGE-modified LDL may be the result of uptake of lipid peroxides and their subsequent reaction with circulating lipoproteins, without having to invoke the assimilation of AGEs from the diet.

Studies in experimental models have demonstrated an important relationship between high dietary intake of AGEs and development or progression of diabetes-related tissue damage, both vascular and renal complications. There is one report to suggest that exposure to high levels of exogenous AGEs may directly contribute to the development of albuminuria and atherosclerosis in otherwise normal animals [221]. In both contexts, end-organ damage was prevented by dietary restriction of AGEs. More recently, a significant suppression of circulating levels of vascular disease markers (e.g., adhesion molecules) as well as of inflammatory mediators has been associated with restriction of dietary AGEs in patients with renal disease [222].

It seems likely that modulation of AGEs in the diet may ultimately provide an important adjunct to interventions directed toward preventing the accumulation of AGEs in diabetes and other degenerative conditions, analogous to the role of a low cholesterol diet in the setting of concomitant statin therapy to reduce hyperlipidemia [223]. These dietary

changes may be little different from recommendations currently advocated for a healthy diet such as increased consumption of fresh foods or limited cooking or only brief applications of heat, in the presence of ample water or humidity. Thus, recommendations for a healthy diet currently serve to limit the AGE content of the diet. Such regimens have been reported to decrease the intake of AGEs by more than 50% and reduce circulating AGEs by ~30% within a month [223].

Tobacco smoke is a significant exogenous source of AGEs. The curing and burning of tobacco produces a number of AGE precursors, which may be transferred to blood by inhalation [224]. Certainly, tissue and circulating AGEs are significantly higher in smokers [225]. These findings provide yet one more reason to encourage diabetic and renal patients to quit the smoking habit.

## NOVEL INHIBITORS OF AGE FORMATION

Many other agents have been suggested as possible inhibitors of AGE formation (Table 1).

### Carnosine

Carnosine is a dipeptide that contains a  $\beta$ -alanine, whose carboxyl group is amide-linked to histidine ( $\beta$ -alanyl-L-histidine) (Fig. (10)). It is present in the brain and a number of other cell types, where it is known to play a role in the response to cellular damage. More recently it has been demonstrated that this endogenous product may also inhibit AGE formation [226] and prevent protein modification by carbonyl compounds such as glyceraldehyde 3-phosphate [227] and methylglyoxal [228]. The mechanism of carnosine's actions to inhibit advanced glycation are poorly understood. Carnosine is known to react with carbohydrates [228] reactive oxygen species [229] and oxidized lipids [230]. The primary amine clearly has a role in these actions *in vitro*, potentially by providing an alternative site for modification as well as scavenging reactive carbonyls. For example, unconjugated histidine and  $\beta$ -alanine have demonstrable AGE-inhibitory activity, though N-acetylated

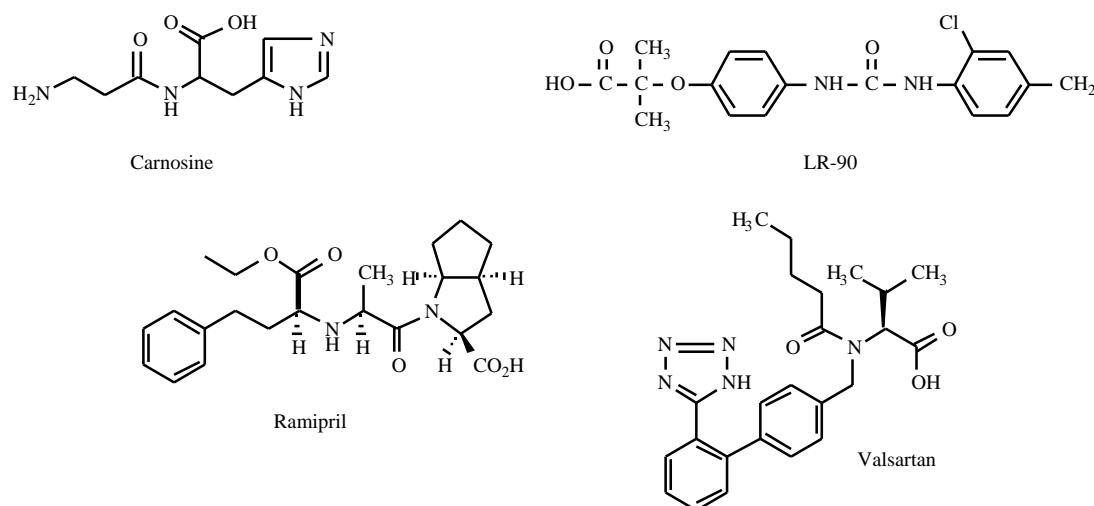


Fig. (10). The structure of carnosine, LR-90, ramipril, and valsartan.



histidine is inactive [231]. However, histidine has greater activity, suggesting the histidine imidazolium group may provide additional action, possibly by stabilizing the adduct formed at primary amine or by metal chelation. This is further supported by the finding that methylation at the N-1 position of imidazole abolished anti-cross linking activity of histidine and diminished anti-crosslinking activity of carnosine [232].

### Alpha-Lipoic Acid

Alpha-lipoic acid (lipoate) has recently been suggested as a potential therapeutic agent for diabetes-induced complications (Fig. (10)). *In vitro* studies in which proteins have been incubated with 0.5-1M glucose, demonstrate that lipoate is able to prevent the AGE-modification including fluorescence and CML. The role of this action in their apparent therapeutic efficacy *in vivo* remains to be established.

### The LR Compounds

Recently two new classes of aromatic compounds have been proposed as potential AGE inhibitors including aryl- (and heterocyclic) ureido and aryl (and heterocyclic) carboxamido phenoxyisobutyric acids, and benzoic acid derivatives and related compounds [232]. One of these compounds (LR-90, methylene bis [4, 4'-(2-chlorophenyl)-ureidophenoxyisobutyric acid]) (Fig. (10)) has now been shown to prevent the progression of nephropathy in streptozotocin-diabetic rats [233]. Their exact mechanism of action remains to be established, although it is clear that many of the LR compounds are also potent metal chelators, some being able to interact with reactive carbonyl compounds such as methylglyoxal.

### Transition Metal Chelators

Metal-catalyzed glucose auto-oxidation and oxidation of glycosylated residues are potent sources of AGE intermediates. Transition metal chelators including penicillamine, trientine and desferrioxamine are able to inhibit the formation of AGEs *in vitro* [234, 235]. More recently, trientine has been shown to reduce myocardial AGE accumulation in experimental diabetes [236]. That many of the established AGE inhibitors also have metal chelation properties, supports the hypothesis that metal catalyzed oxidation is an important component of AGE-mediated damage as well as in the formation of AGEs *in vivo*. However, the chelation of free metals alone is unlikely to explain the actions of these agents in diabetes. Although the generation of AGEs in the test tube appears to be critically dependent on the presence of trace amounts of metal ions, the role for chelators in reducing AGE accumulation in diabetes remains to be established. Moreover, reduced levels of copper or iron may be paradoxically associated with increased AGE formation and accelerated atherosclerosis in experimental models [237].

### Oral Hypoglycemics

In addition to lowering glucose levels, both metformin and the thiazolidinedione hypoglycemics are able to inhibit the formation of AGEs *in vitro* [238]. In patients with type 2 diabetes, high dose metformin treatment is able to reduce methylglyoxal levels possibly by trapping of trapping of

dicarbonyls in a manner similar to aminoguanidine [239]. The mechanism by which PPARs might reduce AGE remains to be established, although they have been shown to have antioxidant and lipid lowering activities that may be relevant to AGE formation.

### Blockade of the RAS

As noted above, blockade of the RAS using ACE inhibitors or ARAs results significantly in attenuating the formation and accumulation of AGEs in experimental diabetes [240]. Recently, similar results have been demonstrated in patients with hypertension [241]. The mechanism of the ACE-induced inhibition of AGE formation is yet to be established. ACE inhibitors can attenuate the formation of reactive oxygen species, thereby reducing AGEs formed as a result of glycoxidation. In experimental diabetes, both aminoguanidine and the ACE inhibitor, ramipril (Fig. (10)) [58], reduce levels of the oxidative stress marker, nitrotyrosine by a similar amount. In addition, ACE inhibition may directly reduce AGE formation, as simultaneous incubation of ACE inhibitors or ARAs with glucose and protein prevents the *in vitro* formation of AGEs, possibly the result of chelation of transition metal ions [166]. Attenuation of renal injury and augmented renal blood flow may also serve to reduce the accumulation of AGEs in diabetes. Whatever the mechanism of action, the utility of blockade of the RAS appears synergistic to that of inhibition of AGE accumulation with aminoguanidine, suggesting that the major mechanism of action of these agents is primarily hemodynamic. Nonetheless, they clearly form a pivotal component or vasculoprotective therapy in diabetes.

### Antioxidants

Increased oxidative stress associated with diabetes is one of the key factors promoting the formation and accumulation of AGEs in the vasculature. AGE deposited in the arterial wall also generate free radicals capable of oxidizing vascular wall lipids and accelerate atherogenesis in diabetes. Despite these known facts, antioxidant therapy has proved largely ineffective in reducing AGE levels *in vivo*. For example, high dose vitamin E was unable to prevent increased AGEs in the femoral artery after 12-weeks of diabetes [242]. More recently, aspirin, benzoic acid inositol and probucol have been found to have significant AGE-inhibition *in vitro*, possibly by primarily preventing the autooxidative pathways of AGE formation [239].

### CONCLUDING REMARKS

By 2025, there will be nearly 300 million people with diabetes worldwide. In the absence of population-based approaches to prevent this 'epidemic', these individuals will require some form of intervention to prevent irreversible end-organ damage. In particular, the additional burden of CVD will overwhelm health systems currently struggling under the tide of diabetic complications. Present management practices are based around the assumption that the best way to reduce the risk of microvascular complications is to achieve optimal glycemic control. However, it needs to be pointed out that patients receiving intensive therapy designed to achieve glycemic control still develop CVD. While

glycemic control is undoubtedly a valuable and important treatment, exogenous insulin therapy or oral hypoglycemics cannot match the kinetics of a healthy endocrine system. It seems likely that, short of successful islet cell transplantation/regeneration, hyperglycemia is unavoidable in patients with diabetes. Furthermore, it is clear that continuous florid hyperglycemia is not necessarily required for vascular dysfunction, which may persist in patients with diabetes even after euglycemia is achieved through aggressive insulin therapy. In addition, it may be that intermittent signals in the form of transient elevations in filtered glucose are sufficient to induce vascular damage and contribute to premature atherosclerosis. Such transient (predominantly post-prandial) elevations of glucose may be observed years before the onset of type 2 diabetes, and contribute to vascular damage before a diagnosis of diabetes is even made. If hyperglycemia cannot be effectively prevented, the only way to impede CVD in patients with diabetes will be to interrupt the pathways that lead from hyperglycemia to vascular injury. AGEs clearly represent one such pathway.

This review has discussed only a handful of therapeutic agents that may be useful in the management of diabetes. In the future, it is likely that more potent and specific agents will emerge to counter the accumulation of AGEs in diabetes. In the meanwhile, the best way to impede CVD in diabetes is to prevent microvascular disease and, in particular, nephropathy [243]. Below the age of 50 years, the excess of mortality from CVD is almost entirely confined to patients with diabetic nephropathy [244]. AGEs independently contribute to the development of nephropathy, as inhibitors of advanced glycation are able to retard the development of renal disease without directly influencing glycemic control. In addition, direct *in vivo* exposure to AGEs in rats is able to generate renal lesions similar to those seen in diabetes [245]. However, renal damage *per se* also contributes to the accumulation of AGEs, since the kidney is the major site of catabolism and clearance of AGEs and their precursors [112]. To this end, optimal metabolic and blood pressure control, achieved early in the disease and sustained indefinitely, remains the best recourse to prevent the accumulation of AGEs until more specific agents become a clinical reality. And even then, it is likely that these agents will need to be combined with other metabolic and hemodynamic interventions for maximal efficacy.

## REFERENCES

- Garcia, M.J., McNamara, P.M., Gordon, T. and Kannel, W.B. (1974) *Diabetes*, **23**, 105-111.
- Cooper, M.E. and Johnston, C.I. (2000) *JAMA*, **283**, 3177-3179.
- Brownlee, M. (2001) *Nature*, **414**, 813-820.
- Soulis-Liparota, T., Cooper, M.E., Dunlop, M. and Jerums, G. (1995) *Diabetologia*, **38**, 387-394.
- Hammes, H., Martin, S., Federlin, K., Geisen, K. and Brownlee, M. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 11555-11558.
- Forbes, J.M., Yee, L.T., Thallas, V., Lassila, M., Candido, R., Jandeleit-Dahm, K.A., Thomas, M.C., Burns, W.C., Deemer, E.K., Thorpe, S.M., Cooper, M.E. and Allen, T.J. (2004) *Diabetes*, **53**(7), 1813-23.
- Soulis, T., Sastra, S., Thallas, V., Mortensen, S.B., Wilken, M., Clausen, J.T., Bjerrum, O.J., Petersen, H., Lau, J., Jerums, G., Boel, E. and Cooper, M.E. (1999) *Diabetologia*, **42**, 472-479.
- Forbes, J.M., Cooper, M.E., Oldfield, M.D. and Thomas, M.C. (2003) *J. Am. Soc. Nephrol.*, **14** (8 Suppl. 3), S254-8.
- Vlassara, H. (2001) *Diabetes Metab. Res. Rev.*, **17**, 436-443.
- Soulis-Liparota, T., Cooper, M., Papazoglou, D., Clarke, B. and Jerums, G. (1991) *Diabetes*, **40**, 1328-1334.
- Lin, R.Y., Choudhury, R.P., Cai, W., Lu, M., Fallon, J.T., Fisher, E.A. and Vlassara, H. (2003) *Atherosclerosis*, **168**, 213-220.
- Szweda, P.A., Friguet, B. and Szweda, L.I. (2002) *Free Radic. Biol. Med.*, **33**, 29-36.
- Fu, M.X., Wells-Knecht, K.J., Blackledge, J.A., Lyons, T.J., Thorpe, S.R. and Baynes, J.W. (1994) *Diabetes*, **43**, 676-683.
- Yaylayan, V.A., Keyhani, A. and Huygues-Despointes, A. (1998) *Adv. Exp. Med. Biol.*, **434**, 237-244.
- Hayase, F., Shibuya, T., Sato, J. and Yamamoto, M. (1996) *Biosci. Biotechnol. Biochem.*, **60**, 1820-1825.
- Reddy, S., Bichler, J., Wells-Knecht, K.J., Thorpe, S.R. and Baynes, J.W. (1995) *Biochemistry*, **34**, 10872-10878.
- Moreau, R., Nguyen, B.T., Doneanu, C.E. and Hagen, T.M. (2005) *Biochem. Pharmacol.*, **69**(1), 29-40.
- Lyons, T.J., Bailie, K.E., Dyer, D.G., Dunn, J.A. and Baynes, J.W. (1991) *J. Clin. Invest.*, **87**(6), 1910-5.
- Beisswenger, P.J., Makita, Z., Curphey, T.J., Moore, L.L., Jean, S., Brinck-Johnsen, T., Bucala, R. and Vlassara, H. (1995) *Diabetes*, **44**, 824-829.
- Thornalley, P.J., Langborg, A. and Minhas, H.S. (1999) *Biochem. J.*, **344**(1), 109-116.
- Bai, P., Phua, K., Hardt, T., Cernadas, M. and Brodsky, B. (1992) *Connect. Tissue Res.*, **28**, 1-12.
- Haitoglou, C.S., Tsilibary, E.C., Brownlee, M. and Charonis, A.S. (1992) *J. Biol. Chem.*, **267**, 12404-12407.
- Silbiger, S., Crowley, S., Shan, Z., Brownlee, M., Satriano, J. and Schlondorff, D. (1993) *Kidney Int.*, **43**, 853-864.
- Mott, J.D., Khalifah, R.G., Nagase, H., Shield, C.F. 3rd., Hudson, J.K. and Hudson, B.G. (1997) *Kidney Int.*, **52**, 1302-1312.
- Nyengaard, J.R., Chang, K., Berhorst, S., Reiser, K.M., Williamson, J.R. and Tilton, R.G. (1997) *Diabetes*, **46**, 94-106.
- Dyer, D.G., Dunn, J.A., Thorpe, S.R., Bailie, K.E., Lyons, T.J., McCance, D.R. and Baynes, J.W. (1993) *J. Clin. Invest.*, **91**, 2463-2469.
- Soulis, T., Thallas, V., Youssef, S., Gilbert, R.E., McWilliam, B., Murray-McIntosh, R.P. and Cooper, M.E. (1997) *Diabetologia*, **40**, 619-628.
- Miura, J., Yamagishi, S., Uchigata, Y., Takeuchi, M., Yamamoto, H., Makita, Z. and Iwamoto, Y. (2003) *J. Diabetes Complicat.*, **17**, 16-21.
- Karachalias, N., Babaei-Jadidi, R., Ahmed, N. and Thornalley, P.J. (2003) *Biochem. Soc. Trans.*, **31**(Pt 6), 1423-5.
- Ahmed, N., Dobler, D., Dean, M. and Thornalley, P.J. (2004) *J. Biol. Chem.*
- Fan, X., Subramaniam, R., Weiss, M.F. and Monnier, V.M. (2003) *Arch. Biochem. Biophys.*, **409**(2), 274-86.
- Stitt, A.W., He, C., Friedman, S., Scher, L., Rossi, P., Ong, L., Founds, H., Li, Y.M., Bucala, R. and Vlassara, H. (1997) *Mol. Med.*, **3**, 617-627.
- Sell, D.R., Lapolla, A., Odetti, P., Fogarty, J. and Monnier, V.M. (1992) *Diabetes*, **41**, 1286-1292.
- Monnier, V.M., Bautista, O., Kenny, D., Sell, D.R., Fogarty, J., Dahms, W., Cleary, P.A., Lachin, J. and Genuth, S. (1999) *Diabetes*, **48**, 870-880.
- Cipollone, F., Iezzi, A., Fazio, M., Zucchelli, M., Pini, B., Cuccurullo, C., De Cesare, D., De Blasis, G., Muraro, R., Bei, R., Chiarelli, F., Schmidt, A.M., Cuccurullo, F. and Mezzetti, A. (2003) *Circulation*, **108**, 1070-1077.
- Sugiyama, S., Miyata, T., Ueda, Y., Tanaka, H., Maeda, K., Kawashima, S., Van Ypersele, de Strihou, C. and Kurokawa, K. (1998) *J. Am. Soc. Nephrol.*, **9**, 1681-1688.
- Yim, M.B., Yim, H.S., Lee, C., Kang, S.O. and Chock, P.B. (2001) *Ann. N. Y. Acad. Sci.*, **928**, 48-53.
- Hogan, M., Cerami, A. and Bucala, R. (1992) *J. Clin. Invest.*, **90**, 1110-1115.
- Dart, A.M. and Kingwell, B.A. (2001) *J. Am. Coll. Cardiol.*, **37**, 975-984.
- Laurent, S., Boutouyrie, P., Asmar, R., Gautier, I., Laloux, B., Guize, L., Ducimetiere, P. and Benetos, A. (2001) *Hypertension*, **37**, 1236-1241.
- Boutouyrie, P., Tropeano, A.I., Asmar, R., Gautier, I., Benetos, A., Lacolley, P. and Laurent, S. (2002) *Hypertension*, **39**, 10-15.
- Meaume, S., Benetos, A., Henry, O.F., Rudnichi, A. and Safar, M.E. (2001) *Arterioscler. Thromb. Vasc. Biol.*, **21**, 2046-2050.

- [43] Watanabe, H., Ohtsuka, S., Kakihana, M. and Sugishita, Y. (1993) *J. Am. Coll. Cardiol.*, **21**, 1497-1506.
- [44] Kelly, R.P., Tunin, R. and Kass, D.A. (1992) *Circ. Res.*, **71**, 490-502.
- [45] Knott, H.M., Brown, B.E., Davies, M.J. and Dean, R.T. (2003) *Eur. J. Biochem.*, **270**, 3572-3582.
- [46] Candido, R., Jandeleit-Dahm, K.A., Cao, Z., Nesteroff, S.P., Burns, W.C., Twigg, S.M., Dilley, R.J., Cooper, M.E. and Allen, T.J. (2002) *Circulation*, **106**(2), 246-53.
- [47] Neeper, M., Schmidt, A.M., Brett, J., Yan, S.D., Wang, F., Pan, Y.C., Elliston, K., Stern, D. and Shaw, A. (1992) *J. Biol. Chem.*, **267**, 14998-15004.
- [48] Li, Y., Mitsuhashi, T., Wojciechowicz, D., Shimizu, N., Li, J., Stitt, A., He, C., Banerjee, D. and Vlassara, H. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 11047-11052.
- [49] Mitsuhashi, T., Li, Y.M., Fishbane, S. and Vlassara, H. (1997) *J. Clin. Invest.*, **100**, 847-854.
- [50] Ohgami, N., Miyazaki, A., Sakai, M., Kuniyasu, A., Nakayama, H. and Horiuchi, S. (2003) *J. Atheroscler. Thromb.*, **10**, 1-6.
- [51] McRobert, E.A., Gallicchio, M., Jerums, G., Cooper, M.E. and Bach, L.A. (2003) *J. Biol. Chem.*, **278**, 25783-25789.
- [52] Hofmann, M.A., Drury, S., Fu, C., Qu, W., Taguchi, A., Lu, Y., Avila, C., Kambham, N., Bierhaus, A., Nawroth, P., Neurath, M.F., Slattey, T., Beach, D., McClary, J., Nagashima, M., Morser, J., Stern, D. and Schmidt, A.M. (1999) *Cell*, **97**, 889-901.
- [53] Valencia, J.V., Weldon, S.C., Quinn, D., Kiers, G.H., DeGroot, J., TeKoppele, J.M. and Hughes, T.E. (2004) *Anal. Biochem.*, **324**, 68-78.
- [54] Valencia, J.V., Mone, M., Koehne, C., Rediske, J. and Hughes, T.E. (2004) *Diabetologia*, **47**(5), 844-52.
- [55] Abordo, E.A., Westwood, M.E. and Thornalley, P.J. (1996) *Immunol. Lett.*, **53**(1), 7-13.
- [56] Hammes, H.P., Hoerauf, H., Alt, A., Schleicher, E., Clausen, J.T., Bretzel, R.G. and Laqua, H. (1999) *Invest. Ophthalmol. Vis. Sci.*, **40**, 1855-1859.
- [57] Sun, M., Yokoyama, M., Ishiwata, T. and Asano, G. (1998) *Int. J. Exp. Pathol.*, **79**(4), 207-22.
- [58] Osicka, T.M., Yu, Y.X., Panagiotopoulos, S., Clavant, S.P., Kiriazis, Z., Pike, R.N., Pratt, L.M., Russo, L.M., Kemp, B.E., Comper, W.D. and Jerums, G. (2000) *Diabetes*, **49**, 87-93.
- [59] Rumble, J.R., Cooper, M.E., Soulis, T., Cox, A., Wu, L., Youssef, S., Jasik, M., Jerums, G. and Gilbert, R.E. (1997) *J. Clin. Invest.*, **99**, 1016-1027.
- [60] Twigg, S.M., Chen, M.M., Joly, A.H., Chakrapani, S.D., Tsubaki, J., Kim, H.S., Oh, Y. and Rosenfeld, R.G. (2001) *Endocrinology*, **142**, 1760-1769.
- [61] Kelly, D.J., Gilbert, R.E., Cox, A.J., Soulis, T., Jerums, G. and Cooper, M.E. (2001) *J. Am. Soc. Nephrol.*, **12**, 2098-2107.
- [62] Miyata, T., Inagi, R., Iida, Y., Sato, M., Yamada, N., Oda, O., Maeda, K. and Seo, H. (1994) *J. Clin. Invest.*, **93**, 521-528.
- [63] Vlassara, H., Fuh, H., Donnelly, T. and Cybulsky, M. (1995) *Mol. Med.*, **1**, 1076-1551.
- [64] Bierhaus, A., Schiekofer, S., Schwaninger, M., Andrassy, M., Humpert, P.M., Chen, J., Hong, M., Luther, T., Henle, T., Klötting, I., Morcos, M., Hofmann, M., Tritschler, H., Weigle, B., Kasper, M., Smith, M., Perry, G., Schmidt, A.M., Stern, D.M., Haring, H.U., Schleicher, E. and Nawroth, P.P. (2001) *Diabetes*, **50**, 2792-2808.
- [65] Kirstein, M., Aston, C., Hintz, R. and Vlassara, H. (1992) *J. Clin. Invest.*, **90**, 439-446.
- [66] Brownlee, M., Vlassara, H. and Cerami, A. (1984) *Diabetes*, **33**, 532-535.
- [67] Higashi, T., Sano, H., Saishoji, T., Ikeda, K., Jinnouchi, Y., Kanzaki, T., Morisaki, N., Rauvala, H., Shichiri, M. and Horiuchi, S. (1997) *Diabetes*, **46**(3), 463-72.
- [68] Seftel, A.D., Vaziri, N.D., Ni, Z., Razmjouei, K., Fogarty, J., Hampel, N., Polak, J., Wang, R.Z., Ferguson, K., Block, C. and Haas, C. (1997) *Urology*, **50**, 1016-1026.
- [69] Huang, J.S., Guh, J.Y., Chen, H.C., Hung, W.C., Lai, Y.H. and Chuang, L.Y. (2001) *J. Cell Biochem.*, **81**, 102-113.
- [70] Li, H.J., Wang, W.S., Huang, X.R., Oldfield, M., Schmidt, A., Cooper, M.E. and Lan, H.Y. (2004) *Am. J. Pathol.*, **164**(4), 1389-97.
- [71] Basta, G., Schmidt, A.M. and De Caterina, R. (2004) *Cardiovasc. Res.*, **63**(4), 582-92.
- [72] Kim, Y.S., Kim, B.C., Song, C.Y., Hong, H.K., Moon, K.C. and Lee, H.S. (2001) *J. Lab. Clin. Med.*, **138**, 59-68.
- [73] Oldfield, M.D., Bach, L.A., Forbes, J.M., Nikolic-Paterson, D., McRobert, A., Thallas, V., Atkins, R.C., Osicka, T., Jerums, G. and Cooper, M.E. (2001) *J. Clin. Invest.*, **108**, 1853-1863.
- [74] Twigg, S.M., Cao, Z., McLennan, S.V., Burns, W.C., Brammar, G., Forbes, J.M., Cooper, M.E. (2002) *Endocrinology*, **143**, 4907-4915.
- [75] Zaoui, P., Cantin, J.F., Alimardani-Bessette, M., Monier, F., Halimi, S., Morel, F. and Cordonnier, D. (2000) *Diabetes Metab.*, **26** (Suppl. 4), 25-29.
- [76] McLennan, S.V., Martell, S.K. and Yue, D.K. (2002) *Diabetes*, **51**, 2612-2618.
- [77] Yonekura, H., Yamamoto, Y., Sakurai, S., Petrova, R.G., Abedin, M.J., Li, H., Yasui, K., Takeuchi, M., Makita, Z., Takasawa, S., Okamoto, H., Watanabe, T. and Yamamoto, H. (2003) *Biochem. J.*, **370**, 1097-1109.
- [78] Bucciarelli, L.G., Wendt, T. and Qu, W. (2002) *Circulation*, **106**(22), 2827-2835.
- [79] Schlueter, C., Hauke, S., Flohr, A.M., Rogalla, P., Bullerdiek, J. (2003) *Biochim. Biophys. Acta*, **1630**, 1-6.
- [80] Pettersson-Fernholm, K., Forsblom, C., Hudson, B.I., Perola, M., Grant, P.J. and Groop, P.H. (2003) *Diabetes*, **52**(3), 891-4.
- [81] He, J., Ogden, L.G., Bazzano, L.A., Vupputuri, S., Loria, C. and Whelton, P.K. (2001) *Arch. Intern. Med.*, **161**, 996-1002.
- [82] McAlister, F.A., Teo, K.K., Taher, M., Montague, T.J., Humen, D., Cheung, L., Kiai, M., Yim, R. and Armstrong, P.W. (1999) *Am. Heart J.*, **138**, 87-94.
- [83] Dries, D.L., Sweitzer, N.K., Drazner, M.H., Stevenson, L.W. and Gersh, B.J. (2001) *J. Am. Coll. Cardiol.*, **38**, 421-428.
- [84] Norton, G.R., Candy, G. and Woodiwiss, A.J. (1996) *Circulation*, **93**, 1905-1912.
- [85] Petrova, R., Yamamoto, Y., Muraki, K., Yonekura, H., Sakurai, S., Watanabe, T., Li, H., Takeuchi, M., Makita, Z., Kato, I., Takasawa, S., Okamoto, H., Imaizumi, Y. and Yamamoto, H. (2002) *J. Mol. Cell. Cardiol.*, **34**(10), 1425-31.
- [86] Candido, R., Forbes, J.M., Thomas, M.C., Thallas, V., Dean, R.G., Burns, W.C., Tikellis, C., Ritchie, R.H., Twigg, S.M., Cooper, M.E. and Burrell, L.M. (2003) *Circ. Res.*, **92**, 785-792.
- [87] Berg, T.J., Snorgaard, O., Faber, J., Torjesen, P.A., Hildebrandt, P., Mehlsen, J. and Hanssen, K.F. (1999) *Diabetes Care*, **22**(7), 1186-90.
- [88] Forbes, J.M., Cooper, M.E., Thallas, V., Burns, W.C., Thomas, M.C., Brammar, G.C., Lee, F., Grant, S.L., Burrell, L.A., Jerums, G. and Osicka, T.M. (2002) *Diabetes*, **51**, 3274-3282.
- [89] Christ, M., Bauersachs, J., Liebetrau, C., Heck, M., Gunther, A. and Wehling, M. (2002) *Diabetes*, **51**, 2648-2652.
- [90] Niwa, T. and Tsukushi, S. (2001) *Kidney Int. Suppl.*, **78**, S37-41.
- [91] Fujii, J., Myint, T., Okado, A., Kaneto, H. and Taniguchi, N. (1996) *Nephrol. Dial. Transplant.*, **11**, 34-40.
- [92] Lal, M.A., Brismar, H., Eklof, A.C. and Aperia, A. (2002) *Kidney Int.*, **61**, 2006-2014.
- [93] Baynes, J.W. and Thorpe, S.R. (1999) *Diabetes*, **48**, 1-9.
- [94] Bucala, R., Makita, Z., Vega, G., Grundy, S., Koschinsky, T., Cerami, A. and Vlassara, H. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 9441-9445.
- [95] Degenhardt, T.P., Alderson, N.L., Arrington, D.D., Beattie, R.J., Basgen, J.M., Steffes, M.W., Thorpe, S.R. and Baynes, J.W. (2002) *Kidney Int.*, **61**, 939-950.
- [96] MRC/BHF Heart Protection Study Group. (2002) *Lancet*, **360**, 7-22.
- [97] Hollenberg, N.K., Price, D.A., Fisher, N.D., Lansang, M.C., Perkins, B., Gordon, M.S., Williams, G.H. and Laffel, L.M. (2003) *Kidney Int.*, **63**, 172-178.
- [98] Jandeleit-Dahm, K. and Cooper, M.E. (2002) *Curr. Opin. Nephrol. Hypertens.*, **11**, 221-228.
- [99] Candido, R., Allen, T.J., Lassila, M., Cao, Z., Thallas, V., Cooper, M.E. and Jandeleit-Dahm, K.A. (2004) *Circulation*, **109**(12), 1536-42.
- [100] Fukami, K., Ueda, S., Yamagishi, S., Kato, S., Inagaki, Y., Takeuchi, M., Motomiya, Y., Bucala, R., Iida, S., Tamaki, K., Imaizumi, T., Cooper, M.E. and Okuda, S. (2004) *Kidney Int.*, **66**(6), 2137-47.
- [101] Davis, B.J., Forbes, J.M., Thomas, M.C., Jerums, G., Burns, W.C., Kawachi, H., Allen, T.J. and Cooper, M.E. (2004) *Diabetologia*, **47**(1), 89-97.
- [102] Thornalley, P.J. (2003) *Arch. Biochem. Biophys.*, **419**(1), 31-40.

- [103] Zauder, H.L. (1950) *Proc. Soc. Exp. Biol. Med.*, **74**(3), 598-9.
- [104] Acharya, A.S., Cho, Y.J. and Manjula, B.N. (1988) *Biochemistry*, **27**(12), 4522-9.
- [105] Ihm, S.H., Yoo, H.J., Park, S.W. and Ihm, J. (1999) *Metabolism*, **48**(9), 1141-5.
- [106] Szabo, C., Ferrer-Sueta, G., Zingarelli, B., Southan, G.J., Salzman, A.L. and Radi, R. (1997) *J. Biol. Chem.*, **272**(14), 9030-6.
- [107] Price, D.L., Rhett, P.M., Thorpe, S.R. and Baynes, J.W. (2001) *J. Biol. Chem.*, **276**(52), 48967-72.
- [108] Nilsson, B.O. (1999) *Inflamm. Res.*, **48**(10), 509-15.
- [109] Gugliucci, A. and Menini, T. (2003) *Life Sci.*, **72**(23), 2603-16.
- [110] Misko, T.P., Moore, W.M., Kasten, T.P., Nickols, G., Corbett, J.A., Tilton, R.G., McDaniel, M.L. and Williamson, J.R. and Currie, M.G. (1993) *Eur. J. Pharmacol.*, **233**(1), 119-25.
- [111] Soulis, T., Cooper, M.E., Sastra, S., Thallas, V., Panagiotopoulos, S., Bjerrum, O.J. and Jerums, G. (1997) *Diabetologia*, **40**(10), 1141-51.
- [112] Gugliucci, A. and Bendayan, M. (1996) *Diabetologia*, **39**, 149-160.
- [113] Lewis, B.S. and Harding, J.J. (1990) *Exp Eye Res.*, **50**(5), 463-7.
- [114] Miyoshi, H., Taguchi, T., Sugiura, M., Takeuchi, M., Yanagisawa, K., Watanabe, Y., Miwa, I., Makita, Z. and Koike, T. (2002) *Horm. Metab. Res.*, **34**(7), 371-7.
- [115] Forbes, J.M., Thomas, M.C., Thorpe, S.R., Alderson, N.L. and Cooper, M.E. (2004) *Kidney Int. Suppl.*, **92**, S105-7.
- [116] Brownlee, M., Vlassara, H., Kooney, A., Ulrich, P. and Cerami, A. (1986) *Science*, **232**, 1629-1632.
- [117] Yu, P.H., Wang, M., Deng, Y.L., Fan, H. and Shira-Bock, L. (2002) *Diabetologia*, **45**(9), 1255-62.
- [118] Li, Y.M., Steffes, M., Donnelly, T., Liu, C., Fuh, H., Basgen, J. and Bucala, R. and Vlassara, H. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 3902-3907.
- [119] Boel, E., Selmer, J., Flodgaard, H.J. and Jensen, T. (1995) *J. Diabetes Complicat.*, **9**(2), 104-29.
- [120] Appel, G., Bolton, K. and Freedman, B. (1999) *J. Am. Soc. Nephrol.*, **10**, 153-4.
- [121] Brown, C.D., Zhao, Z.H., Thomas, L.L., deGroof, R. and Friedman, E.A. (2001) *Am. J. Kidney Dis.*, **38**(6), 1414-1420.
- [122] Singleton, C.K. and Martin, P.R. (2001) *Curr. Mol. Med.*, **1**(2), 197-207.
- [123] Booth, A., Khalifah, R.G. and Hudson, B.G. (1996) *Biochem. Biophys. Res. Commun.*, **220**(1), 113-9.
- [124] Booth, A.A., Khalifah, R.G., Todd, P. and Hudson, B.G. (1997) *J. Biol. Chem.*, **272**(9), 5430-7.
- [125] Flora, S.J., Singh, S. and Tandon, S.K. (1989) *J. Int. Med. Res.*, **17**(1), 68-75.
- [126] La Selva, M., Beltramo, E., Pagnozzi, F., Bena, E., Molinatti, P.A., Molinatti, G.M. and Porta, M. (1996) *Diabetologia*, **39**(11), 1263-8.
- [127] Swamy, M.S., Tsai, C., Abraham, A. and Abraham, E.C. (1993) *Exp. Eye Res.*, **56**, 177-185.
- [128] Hammes, H.P., Du, X., Edelstein, D., Taguchi, T., Matsumura, T., Ju, Q., Lin, J., Bierhaus, A., Nawroth, P., Hannak, D., Neumaier, M., Bergfeld, R., Giardino, I. and Brownlee, M. (2003) *Nat. Med.*, **9**(3), 294-9.
- [129] Racker, E., De La Haba, G. and Leder, I.G. (1953) *J. Am. Chem. Soc.*, **75**, 1010-1015.
- [130] Funk, C. (1922) in *The vitamins*. Williams & Wilkins, Baltimore. pp. 388.
- [131] Rathanaswami, R., Pourany, A. and Sundaresan, R. (1991) *Biochem. Int.*, **25**, 577-583.
- [132] Webster, M.J. (1998) *Eur. J. Appl. Physiol. Occup. Physiol.*, **77**(6), 486-91.
- [133] Valerio, G., Franzese, A., Poggi, V., Patrini, C., Laforenza, U. and Tenore, A. (1999) *Acta Diabetol.*, **36**(1-2), 73-6.
- [134] Bakker, S.J., Hoogeveen, E.K., Nijpels, G., Kostense, P.J., Dekker, J.M., Gans, R.O. and Heine, R.J. (1998) *Diabetologia*, **41**(10), 1168-75.
- [135] Poggi, V., Longo, G., De Vizia, B., Andria, G., Rindi, G. and Patrini, C. (1984) *J. Inherit. Metab. Dis.*, **7** (Suppl. 2), 153-154.
- [136] Broderzon, E.A., Lukashik, N.K. and Koltunova, V.I. (1963) *Zdravookhr Beloruss.*, **62**, 17-9.
- [137] Havivi, E., Bar On, H., Reshef, A. and Raz, I. (1991) *Internat. J. Vit. Nutr. Res.*, **61**, 328-333.
- [138] Haugen, H.N. (1964) *Scand. J. Clin. Lab. Investig.*, **16**, 260-266.
- [139] Saito, N., Kimura, M., Kuchiba, A. and Itokawa, Y. (1987) *J. Nutr. Sci. Vitaminol.*, **33**, 421-430.
- [140] Valerio, G., Franzese, A., Poggi, V., Patrini, C., Laforenza, U. and Tenore, A. (1999) *Acta Diabetol.*, **36**, 73-76.
- [141] Hobara, R., Ozawa, K., Okazaki, M. and Yasuhara, H. (1981) *Jpn. J. Pharmacol.*, **31**, 1098-1100.
- [142] Kodentsova, V.M., Vrezhesinskaia, O.A., Sokol'nikov, A.A., Kharitonchik, L.A. and Spirichev, V.B. (1993) *Vopr. Med. Khim.*, **39**, 26-29.
- [143] Shangari, N., Bruce, W.R., Poon, R. and O'Brien, P.J. (2003) *Biochem. Soc. Trans.*, **31**(Pt 6), 1390-3.
- [144] Lubetsky, A., Winaver, J., Seligmann, H., Olchovsky, D., Almog, S., Halkin, H. and Ezra, D. (1999) *J. Lab. Clin. Med.*, **134**(3), 232-7.
- [145] Thomas, M.C., Tsalamandris, C., MacIsaac, R., Medlet, T., Kingwell, B., Cooper, M.E. and Jerums, G. (2004) *Kidney Int.*, **66**(3), 1167-72.
- [146] Hoyumpa, A.M. (1980) *Am. J. Clin. Nutr.*, **33**, 2750-2761.
- [147] Patrini, C., Laforenza, U., Gastaldi, G., Verri, A., Ferrari, G. and Rindi, G. (1996) *J. Physiol. (London)*, **493**, 100S-101S.
- [148] Song, Q. and Singleton C.K. (2002) *B.M.C. Biochem.*, **3**(1), 8.
- [149] Kjos, B. and Seim, S.H. (1977) *Am. J. Clin. Nutr.*, **30**(10), 1591-6.
- [150] Tanaka, T., Kono, T., Terasaki, F., Kintaka, T., Sohmiya, K., Mishima, T. and Kitaura, Y. (2003) *Am. J. Physiol. Heart. Circ. Physiol.*, **285**(4), H1546-53.
- [151] Mall, G., Mattfeldt, T., Mobius, H.J. and Leonhard, R. (1986) *J. Mol. Cell. Cardiol.*, **18**, 635-643.
- [152] Coburn, C.T., Knapp, F.F. Jr., Febbraio, M., Beets, A.L., Silverstein, R.L. and Abumrad, N.A. (2000) *J. Biol. Chem.*, **275**, 32523-32529.
- [153] Barger, P.M. and Kelly, D.P. (1999) *Am. J. Med. Sci.*, **318**, 36-42.
- [154] Friedemann, T.E., Kumeciak, T.C., Keegan, P.K. and Sheft, B.B. (1948) *Gastroenterol.*, **11**(1), 100-114.
- [155] Obrenovich, M.E. and Monnier, V.M. (2003) *Sci. Aging Knowledge Environ.*, **10**, PE6.
- [156] Babaei-Jadidi, R., Karachalias, N., Ahmed, N., Battah, S. and Thornalley, P.J. (2003) *Diabetes*, **52**(8), 2110-20.
- [157] Avena, R., Arora, S., Carmody, B.J., Cosby, K. and Sidawy, A.N. (2000) *Ann Vasc Surg.*, **14**(1), 37-43.
- [158] Beltramo, E., Berrone, E., Buttiglieri, S. and Porta, M. (2004) *Diabetes Metab. Res. Rev.*, **20**(4), 330-6.
- [159] Ascher, E., Gade, P.V., Hingorani, A., Puthukkeril, S., Kallakuri, S., Scheinman, M. and Jacob, T. (2001) *Surgery*, **130**(5), 851-8.
- [160] Lau-Cam, C.A., Thadikonda, K.P. and Kendall, B.F. (1991) *Res. Commun. Chem. Pathol. Pharmacol.*, **73**(2), 197-207.
- [161] Nanbara, S., Tanaka, K., Koide, H., Tanaka, T. and Hayashi, T. (1990) *Diabetes Res. Clin. Pract.*, **9**(2), 109-14.
- [162] Khalifah, R.G., Baynes, J.W. and Hudson, B.G. (1990) *Biochem. Biophys. Res. Commun.*, **257**, 251-258.
- [163] Alderson, N.L., Metz, T.O., Chachich, M.E., Baynes, J.W. and Thorpe, S.R. (2001) *Diabetes*, **50** (Suppl. 2), A172.
- [164] Glomb, M.A. and Pfahler, C. (2002) *J. Biol. Chem.*, **276**, 41638-41647.
- [165] Voziyan, P.A., Metz, T.O., Baynes, J.W. and Hudson, B.G. (2002) *J. Biol. Chem.*, **277**, 3397-3403.
- [166] Miyata, T., van Ypersele de Strihou, C., Ueda, Y., Ichimori, K., Inagi, R., Onogi, H., Ishikawa, N., Nangaku, M. and Kurokawa, K. (2002) *J. Am. Soc. Nephrol.*, **13**, 2478-2487.
- [167] Vasan, S., Zhang, X., Zhang, X., Kapurniotu, A., Bernhagen, J., Teichberg, S., Basgen, J., Wagler, D., Shih, D., Terlecky, I., Bucala, R., Cerami, A., Egan, J. and Ulrich, P. (1996) *Nature*, **382**(6588), 275-8.
- [168] Yang, S.Z., Litchfield, J.E. and Baynes, J.W. (2003) *Arch. Biochem. Biophys.*, **412**(1), 42-6.
- [169] Onorato, J.M., Jenkins, A.J., Thorpe, S.R. and Baynes, J.W. (2000) *J. Biol. Chem.*, **275**, 21177-21184.
- [170] Jain, S.K. and Lim, G. (2001) *Free Radic. Biol. Med.*, **30**, 232-237.
- [171] Shimoi, K., Okitsu, A., Green, M.H., Lowe, J.E., Ohta, T., Kaji, K., Terato, H. and Ide, H. (2001) *Mutat. Res.*, **480-481**, 371-378.
- [172] Suzuki, D., Miyata, T. and Kurokawa, K. (2001) *Contrib. Nephrol.*, **134**, 36-45.
- [173] Degenhardt, T.P., Alderson, N.L., Arrington, D.D., Beattie, R.J., Basgen, J.M., Steffes, M.W., Thorpe, S.R. and Baynes, J.W. (2002) *Kidney Int.*, **61**, 939-950.
- [174] Krentz, A.J. (2003) *Diabetes Obes. Metab.*, **5** (Suppl. 1), S19-27.
- [175] Nagaraj, R.H., Sarkar, P., Mally, A., Biemel, K.M., Lederer, M.O. and Padayatti, P.S. (2002) *Arch. Biochem. Biophys.*, **402**, 110-119.



- [176] Degenhardt, T.P., Alderson, N.L., Arrington, D.D., Beattie, R.J., Basgen, J.M., Steffes, M.W., Thorpe, S.R. and Baynes, J.W. (2002) *Kidney Int.*, **61**, 939-950.
- [177] Zheng, F., Leclercq, B., Elliot, S.J., Striker, L.J. and Striker, G.E. (2002) *J. Am. Soc. Nephrol.*, **13**, 534A.
- [178] Stitt, A., Gardiner, T.A., Alderson, N.L., Canning, P., Frizzell, N., Duffy, N., Boyle, C., Januszewski, A.S., Chachich, M., Baynes, J.W. and Thorpe, S.R. (2002) *Diabetes*, **51**(9), 2826-32.
- [179] Metz, T.O., Alderson, N.L., Thorpe, S.R. and Baynes, J.W. (2003) *Arch. Biochem. Biophys.*, **419**(1), 41-9.
- [180] Jandeliet-Dahm, K., Cooper, M.E., Allen, T.E. (2004) Presented at the 8<sup>th</sup> International congress on the Maillard Reaction., Charleston September.
- [181] Williams, M.E. (2004) *Curr. Diab. Rep.*, **4**(6), 441-6.
- [182] Vasan, S., Foiles, P. and Founds, H. (2003) *Arch. Biochem. Biophys.*, **419**(1), 89-96.
- [183] Vovk, A.I., Murav'eva, I.V. and Parkhomenko, I.M. (2000) *Ukr. Biokhim. Zh.*, **72**(3), 124-32.
- [184] Ulrich, P. and Zhang, X. (1997) *Diabetologia*, **40** (Suppl. 2), S157-9.
- [185] Poinar, H.N., Hofreiter, M., Spaulding, W.G., Martin, P.S., Stankiewicz, B.A., Bland, H., Evershed, R.P., Possnert, G. and Pääbo, S. (1998) *Science*, **281**, 402-406.
- [186] Thornalley, P.J. and Minhas, H.S. (1999) *Biochem. Pharmacol.*, **57**(3), 303-7.
- [187] Ferguson, G.P., VanPatten, S., Bucala, R. and Al-Abed, Y. (1999) *Chem. Res. Toxicol.*, **12**(7), 617-22.
- [188] Mentink, C.J., Hendriks, M., Levels, A.A. and Woffenbutter, B.H. (2002) *Clin. Chim. Acta*, **321**(1-2), 69-76.
- [189] Cooper, M.E., Thallas, V., Forbes, J., Scalbert, E., Sastra, S., Darby, I., Soulis, T. (2000) *Diabetologia*, **43**(5), 660-4.
- [190] Forbes, J.M., Thallas, V., Thomas, M.C., Founds, H.W., Burns, W.C., Jerums, G. and Cooper, M.E. (2003) *FASEB J.*, **17**(12), 176, 2-4.
- [191] Schwedler, S.B., Verbeke, P., Bakala, H., Weiss, M.F., Vilar, J., Depreux, P., Fourmaintraux, E., Striker, L.J. and Striker, G.E. (2001) *Diabetes Obes. Metab.*, **3**(4), 230-9.
- [192] Oturai, P.S., Christensen, M., Rolin, B., Pedersen, K.E., Mortensen, S.B. and Boel, E. (2000) *Metabolism*, **49**(8), 996-1000.
- [193] Woffenbutter, B.H., Boulanger, C.M., Crijns, F.R., Huijberts, M.S., Poitevin, P., Swennen, G.N., Vasan, S., Egan, J.J., Ulrich, P., Cerami, A. and Levy, B.I. (1998) *Proc. Natl. Acad. Sci. USA*, **95**(8), 4630-4.
- [194] Vaitkevicius, P.V., Lane, M., Spurgeon, H., Ingram, D.K., Roth, G.S., Egan, J.J., Vasan, S., Wagle, D.R., Ulrich, P., Brines, M., Wuerth, J.P., Cerami, A. and Lakatta, E.G. (2001) *Proc. Natl. Acad. Sci. USA*, **98**(3), 1171-5.
- [195] Asif, M., Egan, J., Vasan, S., Jyothirmayi, G.N., Masurekar, M.R., Lopez, S., Williams, C., Torres, R.L., Wagle, D., Ulrich, P., Cerami, A., Brines, M. and Regan, T.J. (2000) *Proc. Natl. Acad. Sci. USA*, **97**(6), 2809-13.
- [196] Susic, D., Varagic, J., Ahn, J. and Frohlich, E.D. (2004) *Am. J. Hypertens.*, **17**(4), 328-33.
- [197] Kass, D.A., Shapiro, E.P., Kawaguchi, M., Capriotti, A.R., Scuteri, A., deGroof, R.C. and Lakatta, E.G. (2001) *Circulation*, **104**(13), 1464-1470.
- [198] Liu, J., Masurekar, M.R., Vatner, D.E., Jyothirmayi, G.N., Regan, T.J., Vatner, S.F., Meggs, L.G. and Malhotra, A. (2003) *Am. J. Physiol. Heart. Circ. Physiol.*, **285**(6), H2587-91.
- [199] Kitzman, Zile M., Little, W.C., Hundely, W.G., O'Brien, T.X. and De Groof, R.C. (2003) *Am. J. Geriatr. Cardiol.*, **12**, 125-144.
- [200] Miyata, T., Ueda, Y., Asahi, K., Izuhara, Y., Inagi, R., Saito, A., Van, Ypersele De Strihou, C. and Kurokawa, K. (2000) *J. Am. Soc. Nephrol.*, **11**(9), 1719-25.
- [201] Miyata, T., Ueda, Y., Yamada, Y., Izuhara, Y., Wada, T., Jadoul, M., Saito, A., Kurokawa, K. and van Ypersele de Strihou, C. (1998) *J. Am. Soc. Nephrol.*, **9**, 2349-2356.
- [202] Nakamura, S., Makita, Z., Ishikawa, S., Yasumura, K., Fujii, W., Yanagisawa, K., Kawata, T. and Koike, T. (1997) *Diabetes*, **46**, 895-899.
- [203] Miyata, T., Ueda, Y., Asahi, K., Izuhara, Y., Inagi, R., Saito, A., Van Ypersele, D., Strihou, C. and Kurokawa, K. (2000) *J. Am. Soc. Nephrol.*, **11**(9), 1719-1725.
- [204] Picard, S., Parthasarathy, S., Fruebis, J. and Witztum, J.L. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 6876-6880.
- [205] Tsuchida, K., Makita, Z., Yamagishi, S., Atsumi, T., Miyoshi, H., Obara, S., Ishida, M., Ishikawa, S., Yasumura, K. and Koike, T. (1999) *Diabetologia*, **42**(5), 579-588.
- [206] Yamamoto, Y., Kato, I., Doi, T., Yonekura, H., Ohashi, S., Takeuchi, M., Watanabe, T., Yamagishi, S., Sakurai, S., Takasawa, S., Okamoto, H. and Yamamoto, H. (2001) *J. Clin. Invest.*, **108**(2), 261-8.
- [207] Wada, R., Nishizawa, Y., Yagihashi, N., Takeuchi, M., Ishikawa, Y., Yasumura, K., Nakano, M. and Yagihashi, S. (2001) *Eur. J. Clin. Invest.*, **31**(6), 513-20.
- [208] Miyata, T., Ishikawa, S., Asahi, K., Inagi, R., Suzuki, D., Horie, K., Tatsumi, K. and Kurokawa, K. (1999) *FEBS Lett.*, **445**(1), 202-6.
- [209] Mizutani, K., Ikeda, K., Tsuda, K. and Yamori, Y. (1999) *J. Hypertens.*, **20**(8), 1607-14.
- [210] Schlueter, C., Hauke, S., Flohr, A.M., Rogalla, P. and Bullerdiek, J. (2003) *Biochim. Biophys. Acta*, **1630**(1), 1-6.
- [211] Bucciarelli, L.G., Wendt, T., Qu, W., Lu, Y., Lalla, E., Rong, L.L., Goova, M.T., Moser, B., Kislinger, T., Lee, D.C., Kashyap, Y., Stern, D.M. and Schmidt, A.M. (2002) *Circulation*, **106**(22), 2827-2835.
- [212] Park, L., Raman, K.G., Lee, K.J., Lu, Y., Ferran, L.J., Chow, W.S., Stern, D. and Schmidt, A.M. (1998) *Nat. Med.*, **4**(9), 1025-1031.
- [213] Sakaguchi, T., Sousa, M., Yan, S.D., Yan, S.F., Duda, S., Arnold, B., Nawroth, P.P., Schmidt, A.M., Stern, D.M. and Naka, Y. (2001) *Circulation*, **104** (Suppl. 2), 471 II-522.
- [214] Li, Y.M., Tan, A.X. and Vlassara, H. (1995) *Nat. Med.*, **1**, 1057-1061.
- [215] Li, Y.M., Mitsuhashi, T. and Vlassara, H. (1995) *Diabetes*, **44** (Suppl. 1), 72.
- [216] Zheng, F., Cai, W., Mitsuhashi, T. and Vlassara, H. (2001) *Mol. Med.*, **7**(11), 737-747.
- [217] Goldberg, T., Cai, W., Peppia, M., Dardaine, V., Baliga, B.S., Uribarri, J. and Vlassara, H. (2004) *J. Am. Diet. Assoc.*, **104**(8), 1287-91.
- [218] Friedman, M. (1996) *J. Agric. Food Chem.*, **44**, 631-653.
- [219] Koschinsky, T., He, C.J., Mitsuhashi, T., Bucala, R., Liu, C., Bunting, C., Heitmann, K. and Vlassara, H. (1997) *Proc. Natl. Acad. Sci. USA*, **94**(12), 6474-6479.
- [220] Foerster, A. and Henle, T. (2003) *Biochem. Soc. Trans.*, **31**(Pt 6), 1383-5.
- [221] Zheng, F., He, C., Cai, W., Hattori, M., Steffes, M. and Vlassara, H. (2002) *Diabetes Metab. Res. Rev.*, **18**(3), 224-37.
- [222] Uribarri, J., Peppia, M., Cai, W., Goldberg, T., Lu, M., He, C. and Vlassara, H. (2003) *J. Am. Soc. Nephrol.*, **14**(3), 728-731.
- [223] Fonarow, G.C. and Watson, K.E. (2003) *Am. J. Cardiol.*, **92**(1A), 271-341.
- [224] Cerami, C., Founds, H., Nicholl, I., Mitsuhashi, T., Giordano, D., Vanpatten, S., Lee, A., Al-Abed, Y., Vlassara, H., Bucala, R. and Cerami, A. (1997) *Proc. Natl. Acad. Sci. USA*, **94**(25), 13915-13920.
- [225] Nicholl, I.D., Stitt, A.W., Moore, J.E., Ritchie, A.J., Archer, D.B. and Bucala, R. (1998) *Mol. Med.*, **4**(9), 594-601.
- [226] Swearengen, T.A., Fitzgerald, C. and Seidler, N.W. (1999) *Arch. Toxicol.*, **73**(6), 307-10.
- [227] Seidler, N.W. (2000) *J. Biochem. Mol. Toxicol.*, **14**(4), 215-20.
- [228] Kiss, A.R., Brownson, C., Bertani, M.F., Ruiz, E. and Ferro, A. (2002) *Ann. N. Y. Acad. Sci.*, **959**, 285-94.
- [229] Lee, J.W., Miyawaki, H., Bobst, E.V., Hester, J.D., Ashraf, M. and Bobst, A.M. (1999) *J. Mol. Cell. Cardiol.*, **31**(1), 113-21.
- [230] Carini, M., Aldini, G., Beretta, G., Arlandini, E. and Facino, R.M. (2003) *J. Mass Spectrom.*, **38**(9), 996-1006.
- [231] Hobart, L.J., Seibel, I., Yeargans, G.S. and Seidler, N.W. (2004) *Life Sci.*, **75**(11), 1379-89.
- [232] Rahbar, S. and Figarola, J.L. (2003) *Arch Biochem Biophys.*, **419**(1), 63-79.
- [233] Figarola, J.L., Scott, S., Loera, S., Tessler, C., Chu, P., Weiss, L., Hardy, J. and Rahbar, S. (2003) *Diabetologia*, **46**(8), 1140-52.
- [234] Stevens, A. (1995) *J. Am. Optom. Assoc.*, **66**(12), 744-9.
- [235] Le Guen, C.A., Jones, A.F., Barnett, A.H. and Lunec, J. (1992) *Ann. Clin. Biochem.*, **29** (Pt 2), 184-9.
- [236] Cooper, G.J., Phillips, A.R., Choong, S.Y., Leonard, B.L., Crossman, D.J., Brunton, D.H., Saafi, L., Dissanayake, A.M., Cowan, B.R., Young, A.A., Occleshaw, C.J., Chan, Y.K., Leahy, F.E., Keogh, G.F., Gamble, G.D., Allen, G.R., Pope, A.J., Boyd, P.D., Poppitt, S.D., Borg, T.K., Doughty, R.N. and Baker, J.R. (2004) *Diabetes*, **53**(9), 2501-8.

- [237] Saari, J.T. (2000) *Can. J. Physiol. Pharmacol.*, **78**(10), 848-55.
- [238] Rahbar, S., Natarajan, R., Yerneni, K., Scott, S., Gonzales, N. and Nadler, J.L. (2000) *Clin Chim Acta*, **301**(1-2), 65-77.
- [239] Beisswenger, P.J., Howell, S.K., Touchette, A.D., Lal, S. and Szwergold, B.S. (1999) *Diabetes*, **48**(1), 198-202.
- [240] Forbes, J.M., Thomas, M.C., Thorpe, S.R., Alderson, N.L. and Cooper, M.E. (2004) *Kidney Int. Suppl.*, **92**, S105-7.
- [241] Sebekova, K., Gazdikova, K., Syrova, D., Blazicek, P., Schinzel, R., Heidland, A., Spustova, V. and Dzurik, R. (2003) *J. Hum. Hypertens.*, **17**(4), 265-70.
- [242] Wigg, S.J., Tare, M., Forbes, J., Cooper, M.E., Thomas, M.C., Coleman, H.A., Parkington, H.C. and O'Brien, R.C. (2004) *Diabetologia*, **47**(6), 1038-46.
- [243] Johnsen, K., Andersen, P.K. and Deckert, T. (1985) *Diabetologia*, **28**, 590-596.
- [244] Muhlhauser, I., Sawicki, P.T., Blank, M., Overmann, H., Richter, B. and Berger, M. (2002) *Diabetologia*, **45**, 1490-1497.
- [245] Vlassara, H., Striker, L.J., Teichberg, S., Fuh, H., Li, Y.M. and Steffes, M. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 11704-11708.

Copyright of Current Drug Targets is the property of Bentham Science Publishers Ltd. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.