A Breaker of Advanced Glycation End Products Attenuates Diabetes-Induced Myocardial Structural Changes

Riccardo Candido, Josephine M. Forbes, Merlin C. Thomas, Vicki Thallas, Rachael G. Dean, Wendy C. Burns, Christos Tikellis, Rebecca H. Ritchie, Stephen M. Twigg, Mark E. Cooper, Louise M. Burrell

Abstract—The formation of advanced glycation end products (AGEs) on extracellular matrix components leads to accelerated increases in collagen cross linking that contributes to myocardial stiffness in diabetes. This study determined the effect of the crosslink breaker, ALT-711 on diabetes-induced cardiac disease. Streptozotocin diabetes was induced in Sprague-Dawley rats for 32 weeks. Treatment with ALT-711 (10 mg/kg) was initiated at week 16. Diabetic hearts were characterized by increased left ventricular (LV) mass and brain natriuretic peptide (BNP) expression, decreased LV collagen solubility, and increased collagen III gene and protein expression. Diabetic hearts had significant increases in AGEs and increased expression of the AGE receptors, RAGE and AGE-R3, in association with increases in gene and protein expression of connective tissue growth factor (CTGF). ALT-711 treatment restored LV collagen solubility and cardiac BNP in association with reduced cardiac AGE levels and abrogated the increase in RAGE, AGE-R3, CTGF, and collagen III expression. The present study suggests that AGEs play a central role in many of the alterations observed in the diabetic heart and that cleavage of preformed AGE crosslinks with ALT-711 leads to attenuation of diabetes-associated cardiac abnormalities in rats. This provides a potential new therapeutic approach for cardiovascular disease in human diabetes. (Circ Res. 2003;92:785-792.)

Key Words: diabetes mellitus ■ diabetic cardiomyopathy ■ advanced glycosylation end products ■ growth factors ■ crosslink breaker

Diabetes is an established risk factor for cardiovascular events, especially the development of diabetic heart disease,1 which is associated with coronary heart disease, left ventricular hypertrophy, and cardiac fibrosis.2 These abnormalities contribute to the development of diastolic and systolic dysfunction and ultimately heart failure.2 Although the clinical features of diabetic heart disease have been identified, its pathogenesis and, in particular, the mechanisms underlying the collagen abnormalities in the diabetic heart have not been fully elucidated.

Chronic hyperglycemia contributes to diabetic complications through the formation of advanced glycosylation end products (AGEs), which are irreversibly formed biochemical end products of nonenzymatic glycosylation.3 AGEs may play a key role in the pathogenesis of cardiomyopathy.4 Diabetes produces myocardial stiffness before the development of myocardial fibrosis in association with increased formation of collagen-associated AGEs.4 AGEs can covalently crosslink and biochemically modify protein structure and affect protein functions, particularly collagen.3

Cell surface receptors for AGEs have been identified and via receptor-dependent mechanisms, AGE induction of cytokines and growth factors has been implicated in contributing to diabetic microvascular complications.5,6 The effects that AGEs have on diabetic cardiomyopathy have yet to be determined. Activation of prosclerotic cytokines and stimulation of collagen synthesis may also contribute to diabetic cardiomyopathy. Transforming growth factor (TGF)-β1 is highly expressed in the heart and kidney in experimental diabetes in association with extracellular matrix accumulation,7 and there is increasing evidence that connective tissue growth factor (CTGF) also plays an important role in the development of diabetic renal and cardiovascular complications.8

Inhibitors of AGE formation including aminoguanidine and ALT-946 prevent or retard the development of diabetic nephropathy in rats.9,10 Aminoguanidine also prevents diabetes-induced myocardial stiffness by decreasing the formation of myocardial collagen AGEs.4 The development of thiazolium derivatives that catalytically break existing glucose-derived crosslinks between proteins enables a more direct assessment of the contribution of protein crosslinking to the magnitude of age- or disease-associated changes in...
arterial and ventricular stiffness. The crosslink breaker, 3-phenacyl-4,5-dimethylthiazolium chloride (ALT-711), improves arterial and ventricular function in older rhesus monkeys and vascular compliance in humans. In experimental diabetes, ALT-711 reverses large artery stiffness, whereas the crosslink breaker, N-phenacylthiazolium bromide, prevents vascular AGE accumulation.

Although these data suggest a potential role for crosslink breakers in the prevention and treatment of diabetic cardiovascular complications, the effect of these drugs on diabetic cardiac remodeling has not been examined. The aims of this study were to investigate the molecular mechanisms underlying diabetic cardiomyopathy and to determine the effects of the crosslink breaker ALT-711 on diabetes-associated myocardial disease.

Materials and Methods

Eight-week-old male Sprague-Dawley rats (Austin Biological Research Laboratories, Heidelberg, Victoria, Australia), weighing 200 to 250 g received streptozotocin (Boehringer-Mannheim) at 50 mg/kg. Long-acting insulin (2 U/day Ultratard HM, Novo Industries) was administered to diabetic rats. Sixteen weeks later, diabetic and control animals were randomized (n=15 per group) to receive the crosslink breaker, ALT-711 (Alteon) at 10 mg/kg body weight per day by oral gavage or no treatment for a subsequent 16 weeks. All procedures were in accordance with guidelines set by the Austin Hospital Ethics Committee and the National Health and Medical Research Council of Australia.

Interstitial collagen was measured using Picrosirius red staining, plasma glucose by the glucose oxidase technique, and HbA1c by HPLC. Brain natriuretic peptide (BNP), pro-α1 (III) collagen, pro-α1 (I) collagen, CTGF, TGF-β1, TGF-β1-inducible gene-h3 (β-h3), AG receptor AGE-R3, and receptor for AGE (RAGE) gene expression were analyzed by real-time quantitative RT-PCR using the TaqMan system (ABI Prism 7700, Perkin-Elmer Inc).

To control for variation in the amount of DNA, gene expression of the target sequence was normalized in relation to the expression of an endogenous control, 18S ribosomal RNA (rRNA) (18S rRNA TaqMan Control Reagent kit; ABI Prism 7700, Perkin-Elmer Inc). Primers and Taqman probes for the proteins described above were constructed with the help of Primer Express (ABI Prism 7700, Perkin-Elmer Inc). Results were expressed relative to control LV values, which were arbitrarily assigned a value of 1.

In Situ Hybridization

The site-specific expression of CTGF mRNA was determined by in situ hybridization. Four microm paraffin LV sections were hybridized after digestion with PronaseE at 37°C. The hybridization buffer, containing 2×10⁵ cpm/μL 3²–S-labeled riboprobe, 0.72 mg/mL yeast RNA, 50% deionized formamide, 100 mMol/L DTT, 10% dextran sulfate, 0.3 mol/L NaCl, 10 mmol/L NaHPO₄, 10 mmol/L Tris HCl (pH 7.5), 5 mmol/L EDTA (pH 8.0), 0.02% bovine serum albumin (BSA), 0.02% Ficoll 400, and 0.02% polyvinyl pyridoline (PVP), was added to each section and incubated at 60°C overnight. Slides were exposed to BioMax MR film (Kodak Company) for 3 to 5 days. Slides were coated in Amersham LM-1 Emulsion for 2 to 4 weeks according to the autoradiography results.

Immunohistochemistry

Four-micron paraffin sections of LV were used to stain for collagen I, collagen III, CTGF, carboxymethyllysine, RAGE, and β-h3. The primary antibodies used were a polyclonal goat anti-human type I collagen antibody (SouthernBiotech; diluted 1:100), a polyclonal goat anti-human type III collagen antibody (SouthernBiotech; diluted 1:800), a monoclonal 4G9 anti-carboxymethyllysine antibody, a polyclonal goat anti-human RAGE antibody (kindly donated by Dr Neepo, Merck, West Point, PA; diluted 1:400) and a polyclonal rabbit anti-rat β-h3 antibody (kindly donated by Dr Gibson, Adelaide, SA, Australia; diluted 1:1000).

The staining was visualized by reaction with 3,3′- diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co).

Statistical Analysis

Data were analyzed by ANOVA using Statview V (Brainpower). Comparisons of group means were performed by Fisher’s least significant difference method. Data are shown as mean±SEM, unless otherwise specified. A value of P<0.05 was viewed as statistically significant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=11)</th>
<th>C+ALT-711 (n=10)</th>
<th>Diabetic (n=12)</th>
<th>D+ALT-711 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>710±24</td>
<td>684±23</td>
<td>539±17*</td>
<td>522±11*</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>119±2</td>
<td>117±2</td>
<td>153±5*</td>
<td>149±4*</td>
</tr>
<tr>
<td>Blood glucose, mmol/L</td>
<td>6.3±0.2</td>
<td>6.3±0.1</td>
<td>28.1±1.5*</td>
<td>29.2±1.4*</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>4.5±0.2</td>
<td>5.4±0.2</td>
<td>15.4±0.8*</td>
<td>17.5±0.6*</td>
</tr>
</tbody>
</table>

C indicates control; D, diabetic; SBP, systolic blood pressure; and HbA1c, glycosylated hemoglobin. Data are expressed as mean±SEM. *P<0.05 vs control groups.
Results

Metabolic Parameters and Blood Pressure
Diabetic animals gained less weight than control rats (Table 1). At 32 weeks, diabetic rats had increased plasma glucose and HbA1c values compared with control animals, which was not altered by ALT-711 (Table 1). There was a significant increase in SBP over the 32 weeks in diabetic rats, which was unchanged by ALT-711 (Table 1).

LV Weight and Myocardial BNP Gene Expression
Diabetes was associated with decreased LV weight attributable to reduced body weight in the diabetic animals (Table 2). The ratio of LV to body weight in the diabetic rats was significantly increased compared with controls (Table 2) and was reduced by ALT-711 in the diabetic animals (Table 2). Gene expression of BNP, a marker of cardiac dysfunction, was significantly increased in the diabetic LV (Table 2) and was reduced by ALT-711 (Table 2).

Myocardial Collagen
Diabetes was not associated with changes in myocardial fibrillar collagen, and ALT-711 did not influence myocardial collagen fraction (Table 2). No significant changes were observed in myocardial hydroxyproline concentrations among the four groups studied (Table 2). However, myocardial collagen solubility was significantly decreased in diabetic animals compared with controls (Table 2), which was reduced by ALT-711 (Figure 2A, bottom).

Although no significant differences in cardiac collagen content were noted, diabetic rats had a significant increase in collagen III gene expression compared with nondiabetic rats (Figure 2, top). ALT-711 prevented upregulation of collagen III expression compared with untreated diabetic rats with mRNA levels similar to those observed in control animals (Figure 2, top). Immunohistochemical staining for collagen III demonstrated a similar pattern to that seen with respect to collagen III gene expression. Diabetes was associated with significantly increased LV staining for collagen III compared with control rats (Table 2 and Figures 2A and 2C, bottom), which was reduced by ALT-711 (Table 2 and Figure 2D, bottom).

In contrast to type III collagen, no significant changes in collagen I gene or protein expression were observed in the diabetic LV compared with control rats (Table 2). ALT-711 significantly reduced collagen I gene expression compared with untreated control animals, but this effect was not observed for collagen I protein expression (Table 2).

LV AGEs and AGE Receptors
Myocardial collagen AGE accumulation assessed by collagen fluorescence was significantly increased in diabetic rats compared with controls (Figure 1B), as was immunostaining for AGEs (Figures 1C, 3A, and 3C). In LV from untreated diabetic animals, AGE immunostaining localized predominantly to myocardial interstitium. Treatment of diabetic rats with ALT-711 completely prevented the increases in both myocardial collagen fluorescence and LV AGE immunostaining (Figures 1B, 1C, and 3D).

Although no significant changes were observed in the gene expression for RAGE after 32 weeks of diabetes (Figure 4A), untreated diabetic rats had a significant increase in LV RAGE protein expression with a similar distribution within the heart to that observed for cardiac AGE immunostaining (Table 2 and Figures 3E and 3G). ALT-711 significantly reduced RAGE gene expression compared with untreated control and diabetic animals (Figure 4A) and prevented the increased expression in RAGE protein observed in diabetic animals (Table 2 and Figure 3H). Diabetic rats had a significant increase in gene expression for the receptor AGE-R3 in the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=11)</th>
<th>C+ALT-711 (n=10)</th>
<th>Diabetic (n=12)</th>
<th>D+ALT-711 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV weight, g</td>
<td>1.35±0.04</td>
<td>1.30±0.04</td>
<td>1.12±0.03*</td>
<td>1.02±0.03††</td>
</tr>
<tr>
<td>LV/BW, mg/g</td>
<td>1.86±0.04</td>
<td>1.82±0.03</td>
<td>2.24±0.03*</td>
<td>1.99±0.03††</td>
</tr>
<tr>
<td>BNP gene expression</td>
<td>0.97±0.12</td>
<td>1.00±0.12</td>
<td>1.76±0.29*</td>
<td>1.14±0.10††</td>
</tr>
<tr>
<td>LV interstitial collagen, %</td>
<td>3.09±0.36</td>
<td>2.95±0.33</td>
<td>3.12±0.30</td>
<td>2.67±0.33</td>
</tr>
<tr>
<td>LV HPR concentration, μg/mg</td>
<td>19.3±1.7</td>
<td>17.2±1.1</td>
<td>20.9±1.8</td>
<td>19.4±1.1</td>
</tr>
<tr>
<td>LV collagen III staining, %</td>
<td>28.9±0.4</td>
<td>29.6±0.9</td>
<td>32.3±0.7*</td>
<td>22.5±0.6‡</td>
</tr>
<tr>
<td>LV collagen I mRNA, AU</td>
<td>1.0±0.2</td>
<td>0.4±0.1*</td>
<td>0.6±0.1</td>
<td>0.4±0.1*</td>
</tr>
<tr>
<td>LV collagen I staining, %</td>
<td>27.6±0.6</td>
<td>28.8±0.7</td>
<td>28.5±0.6</td>
<td>27.0±0.5</td>
</tr>
<tr>
<td>LV CTGF staining, %</td>
<td>0.24±0.05</td>
<td>0.16±0.03</td>
<td>1.28±0.10*</td>
<td>0.32±0.06‡</td>
</tr>
<tr>
<td>LV RAGE staining, %</td>
<td>1.0±0.3</td>
<td>5.4±1.2</td>
<td>30.3±3.3*</td>
<td>17.3±4.5‡</td>
</tr>
<tr>
<td>LV TGF-β1 mRNA, AU</td>
<td>1.0±0.1</td>
<td>0.7±0.1</td>
<td>1.1±0.2</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>LV βlg-h3 mRNA, AU</td>
<td>1.0±0.2</td>
<td>0.7±0.1</td>
<td>0.9±0.2</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>LV βlg-h3 staining, %</td>
<td>9.8±0.3</td>
<td>9.9±0.2</td>
<td>10.1±0.2</td>
<td>9.6±0.3</td>
</tr>
</tbody>
</table>

C indicates control; D, diabetic; BW, body weight; BNP, brain natriuretic peptide; HPR, hydroxyproline; AU, arbitrary units; RAGE, receptor for advanced glycation end product; CTGF, connective tissue growth factor; TGF-β1, transforming growth factor-β1; and βlg-h3, β-inducible gene h3. Data are expressed as mean±SEM. *P<0.01 vs control group; †P<0.05 vs diabetic group; and ‡P<0.01 vs diabetic group.
LV compared with nondiabetic animals, and this increase was significantly attenuated by ALT-711 (Figure 4B).

**Prosclerotic Cytokine Expression in the LV**

Diabetic rats had a 2-fold increase in cardiac CTGF gene expression compared with control animals (Figure 5, top). Overexpression of CTGF was confirmed by in situ hybridization in LV from untreated diabetic rats (Figures 5A and 5C, bottom). Consistent with the mRNA findings, increased CTGF protein expression was detected in the LV of diabetic animals (Table 2 and Figure 6C) compared with controls (Table 2 and Figure 6A). CTGF protein expression within the LV was predominantly localized to areas of matrix deposition in the cardiac interstitium (Figure 6C). ALT-711 in diabetic animals was associated with a significant reduction in CTGF gene and protein expression assessed by RT-PCR (Figure 5, top), in situ hybridization (Figures 5D and 5H, bottom), and immunostaining (Figure 6D).

Thirty two weeks of diabetes mellitus failed to alter LV TGF-β, or βig-h3 gene expression (Table 2). ALT-711 in control and diabetic animals did not modify either TGF-β1 or βig-h3 gene expression compared with untreated rats (Table 2). Paralleling the results in βig-h3 gene expression, no significant change in the protein expression of this matrix protein was observed (Table 2).

**Discussion**

In this study, diabetes led not only to marked accumulation of cardiac AGEs, but also to a significant increase in crosslinked collagen and enhanced expression of the fibrillar collagen, type III collagen. Conversely, there were no significant changes in the myocardial collagen content or collagen I gene and protein expression in the diabetic hearts. Cardiac function was not directly assessed in this study, but it is likely that rats with 32 weeks duration of diabetes have cardiac dysfunction, particularly as echocardiographic studies have shown decreased cardiac contractility in this model as early as 6 weeks after the induction of diabetes. Other cardiac abnormalities observed in diabetic rats were increased LV mass and cardiac BNP expression. BNP is released from ventricular myocardium in response to increased wall stress and reflects both systolic and diastolic cardiac dysfunction.
diabetic rats, the development of cardiac dysfunction is also associated with activation of cardiac BNP gene expression. Thus in the present study, the reductions in LV mass and BNP expression with ALT-711 strongly suggest improved cardiac function as a result of intervention with the crosslink breaker. These data are also consistent with the improved cardiac compliance noted previously with ALT-711.12,13 Diabetes is associated with both diastolic and systolic dysfunction. It remains to be determined if the altered cardiac performance in diabetes involves alterations in myocardial collagen structure.2 Multiple mechanisms have been described whereby chronic hyperglycemia contributes to the pathological cardiac remodeling. These include direct effects of elevated glucose on cells, oxidant stress, and nonenzymatic glycation.34,35 AGEs affect the structural components of the extracellular matrix such as collagen and are elevated in serum and tissues of diabetic patients.36,37 Decreased myocardial compliance in diabetic rats is associated with an accumulation of fluorescent AGEs on myocardial collagen and occurs even in the absence of cardiac fibrosis.4 In this study, we confirmed the increase in myocardial AGE formation by collagen fluorescence measurements and extended it also to nonfluorescent AGE moieties as assessed by AGE immunostaining for carboxymethyllysine. Furthermore, cardiac AGE accumulation was associated with a significant decrease in myocardial collagen solubility, an index of increased formation of collagen crosslinks. There was also an increase in the ratio of type III to type I collagen in the diabetic heart. Our findings are in agreement with that observed in diabetic patients, where collagen remodeling occurs mainly as a result of an increase in collagen type III, with no significant differences in the expression of collagen types I and VI between diabetic and control groups.38 An increasing number of AGE receptors have been identified including RAGE, AGE-R1, AGE-R2, galectin-3 (AGE-R3), and the macrophage scavenger receptors.39–41 These proteins are expressed on a wide range of cells including smooth muscle cells, macrophages, endothelial cells, and podocytes. In diabetes, it is postulated that the increase in AGEs activates AGE receptors. Moreover, there is experimental and clinical evidence that RAGE expression is increased in the blood vessels and kidneys in diabetes40,42 and that AGE-R3 is increased in the kidney in diabetic rodents.41 No such data on AGE receptor distribution in the diabetic heart has been previously reported. In our study, a significant increase in AGE-R3 gene expression was detected in the LV of diabetic rats. Diabetes was also associated with a signifi-

Figure 3. Immunohistochemical staining for advanced glycation end product (A through D) and RAGE (E through H) in LV sections from control (A and E), control ALT-711-treated (B and F), diabetic (C and G), and diabetic ALT-711-treated (D and H) rats. Positive staining is shown as brown. Sections are counterstained with hematoxylin. Magnification ×200.

Figure 4. Left ventricular RAGE (A) and AGE-R3 (B) gene expression by RT-PCR in control, control ALT-711-treated (C+ALT-711), diabetic, and diabetic ALT-711-treated (D+ALT-711) rats. Gene expression is expressed relative to the control group, which are arbitrarily designated as 1. *P<0.01 vs control group; †P<0.01 vs diabetic group.
cant increase in left ventricular RAGE protein. The increased expression of RAGE protein in the LV of diabetic animals implies a role for this receptor in mediating AGE-induced myocardial structural alterations. Furthermore, the overexpression of AGE-R3 may influence AGE receptor–mediated events by modifying the function of the AGE-receptor complex. This receptor may also exert direct effects on cardiac remodeling, independently of AGE ligands, by virtue of its adhesive and growth-regulating properties.41

Figure 5. Top, Left ventricular connective tissue growth factor gene expression by RT-PCR in control, control ALT-711–treated (C + ALT-711), diabetic, and diabetic ALT-711–treated (D + ALT-711) rats. Gene expression is expressed relative to the control group, which are arbitrarily designated as 1. *P<0.01 vs control group; †P<0.01 vs diabetic group. Bottom, Representative light-field (left) and darkfield (right) photomicrographs of LV from control (A and E), control ALT-711–treated (B and F), diabetic (C and G), and diabetic ALT-711–treated (D and H) rats labeled in situ with a radiolabeled CTGF riboprobe. Localization of CTGF gene expression is identified in lightfield photomicrographs as dark grains (A through D) and in darkfield photomicrographs as white grains (E through H). Magnification ×200.

Figure 6. Immunohistochemical staining for connective tissue growth factor in LV sections from control (A), control ALT-711–treated (B), diabetic (C), and diabetic ALT-711–treated (D) rats. Positive staining is shown as brown. Sections are counterstained with hematoxylin. Magnification ×200.
has been reported. Of particular interest is CTGF, a profibrotic agent induced by TGF-β that promotes extracellular matrix synthesis and angiogenesis. A role for CTGF in diabetic complications has been recently suggested by in vitro studies that demonstrated an increase in both CTGF gene and protein expression in mesangial cells after exposure to high glucose and in vivo studies assessing CTGF expression in the diabetic rat kidney. Moreover, we have recently demonstrated that CTGF is upregulated in the aorta of diabetic apolipoprotein E-deficient mice and postulated a role for this cytokine in the development and progression of diabetes-associated atherosclerosis. A specific link between AGES and CTGF has been previously described with CTGF upregulated by AGES in both cultured human dermal fibroblasts and mesangial cells. Furthermore, CTGF was a mediator of the induction of the extracellular matrix protein fibronectin by AGES in these cell lines. These authors also reported that in the fibroblast cell line, TGF-β1 did not contribute to AGES induced fibronectin expression. In our study, we observed an increase in both gene and protein expression for CTGF in the LV after 32 weeks of diabetes whereas no changes were observed in the expression of TGF-β1 or the matrix protein, βig-h3, a marker of TGF-β bioactivity. This would suggest that in long-term diabetes, CTGF could independently induce changes in cardiac extracellular matrix proteins. These findings are consistent with studies in experimental myocardial infarction in which CTGF activation has been shown to be involved in cardiac remodeling. Moreover, in transgenic mice over-expressing protein kinase C-β2, CTGF mediates cardiac fibrosis and dysfunction independently from TGF-β activation. Previous studies by our group have demonstrated that inhibitors of AGE formation such as aminoguanidine or ALT-946 retard the development of diabetic nephropathy. It has also been observed that treatment with aminoguanidine in diabetic rats prevents the increase in myocardial stiffness by decreasing the formation of myocardial collagen AGES. An alternative approach to treat AGE-related disease has the development of compounds that cleave preformed AGES and have been described as crosslink breakers. Using one of these agents, N-phenacylthiazolium bromide, we have demonstrated that they are effective in preventing accumulation of AGES in blood vessels and diabetes-associated vascular hypertrophy. The most advanced compound in clinical development is ALT-711, which improves arterial and ventricular function in older rhesus monkeys, reverses large artery stiffness in diabetic rats, and improves total arterial compliance in aged humans. In our study, we have extended these observations to the diabetic heart. Treatment with ALT-711 in diabetic rats significantly reduced the cardiac accumulation of both fluorescent and nonfluorescent AGES and increased collagen solubility to the same level observed in control animals. Furthermore, the crosslink breaker completely prevented the increase in collagen III gene and protein expression. It is postulated that therapy with ALT-711 could prove useful in attenuating cardiac remodeling and in reducing the development of cardiac disease in diabetes.

Acknowledgments

This study was supported by the Juvenile Diabetes Research Foundation and the National Health and Medical Research Council of Australia (NHMRC). Dr Candido is supported by a grant from the University of Trieste, Italy. Dr Thomas is a recipient of an NHMRC Postgraduate Medical Scholarship. Dr Ritchie is supported by the NHMRC as an R. Douglas Wright Fellow. The authors wish to thank Maryann Armstrong and Josefa Schembri for their excellent technical assistance.

References

18. Cefalu WT, Wang ZQ, Bell-Farrow A, Kiger FD, Izlar C. Glycylomeglobin measured by automated affinity HPLC correlates with both


A Breaker of Advanced Glycation End Products Attenuates Diabetes-Induced Myocardial Structural Changes
Riccardo Candido, Josephine M. Forbes, Merlin C. Thomas, Vicki Thallas, Rachael G. Dean, Wendy C. Burns, Christos Tikellis, Rebecca H. Ritchie, Stephen M. Twigg, Mark E. Cooper and Louise M. Burrell

Circ Res. 2003;92:785-792; originally published online March 6, 2003;
doi: 10.1161/01.RES.0000065620.39919.20
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/92/7/785

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/