Advanced Glycation End Products Accumulate in Vascular Smooth Muscle and Modify Vascular but Not Ventricular Properties in Elderly Hypertensive Canines

Brian P. Shapiro, MD*; Theophilus E. Owan, MD, MSc*; Selma F. Mohammed, MBBS; Donna M. Meyer, AA; Lisa D. Mills, BS; Casper G. Schalkwijk, PhD; Margaret M. Redfield, MD

Background—Advanced glycation end products (AGEs) are believed to increase left ventricular (LV) and vascular stiffness, in part via cross-linking proteins. We determined whether and where AGEs were increased in elderly hypertensive nondiabetic dogs and whether an AGE cross-link breaker (ALT-711) improved vascular or ventricular function.

Methods and Results—Elderly dogs with experimental hypertension (old hypertensives [OH]) were randomized to receive ALT-711 (OH+ALT group; n=11; 1 mg/kg PO) or not (OH group; n=11) for 8 weeks. Conscious blood pressure measurements (weekly), echocardiography (week 8), and anesthetized study (week 8) with LV pressure–volume analysis and aortic pressure–dimension and pressure–flow assessment over a range of preloads and afterloads were performed. In LV and aorta from OH, OH+ALT, and young normal dogs, AGE content (immunohistochemistry and Western analysis for N-(carboxymethyl)lysine [CML]) was assessed. Aortic CML content was markedly increased in OH and OH+ALT dogs compared with young normal dogs. CML was localized to aortic and aortic vasa vasorum smooth muscle but not to collagen or elastin. CML was essentially undetectable in young normal, OH, or OH+ALT myocardium but was visible in large vessels in the LV. ALT-711 therapy was associated with lower blood pressure and pulse pressure, decreased systemic vascular resistance, increased aortic distensibility and arterial compliance, and, notably, significant aortic dilatation. Neither LV systolic nor diastolic function was different in OH+ALT versus OH dogs.

Conclusions—In elderly hypertensive canines, AGE accumulation and AGE cross-link breaker effects were confined to the vasculature without evidence of myocardial accumulation or effects. The lack of AGE accumulation in collagen-rich areas suggests that the striking vascular effects may be mediated by mechanisms other than collagen cross-linking.

(Circulation. 2008;118:1002-1010.)

Key Words: aging ■ aorta ■ diastole ■ heart failure ■ hypertension

The aorta dilates with age because of degenerative changes in elastin, the long-lived protein that constitutes 60% of the thoracic aorta. These degenerative changes lead to transfer of stress to less extensible collagenous elements of the aorta and increased aortic stiffness.¹ However, increases in the intrinsic stiffness of the aorta due to processes other than elastin degeneration may also contribute to age-associated vascular dysfunction and development of systolic hypertension, hypertensive heart disease, and heart failure in the elderly.²,³ One mechanism postulated to increase both aortic and left ventricular (LV) diastolic stiffness is protein modification by advanced glycation end products (AGEs).

Clinical Perspective p 1010

AGEs are a diverse group of compounds formed by the reaction of the amino groups on proteins with reducing sugars with subsequent rearrangement and sometimes oxidation, to form stable structures that accumulate on long-lived proteins. AGE formation is accelerated in hyperglycemia, renal failure, and inflammatory conditions.⁴ Some AGEs lead to cross-linking of proteins. It is believed that AGE cross-linking of collagen and elastin increases the tensile strength of these proteins, deters collagen degradation, and increases aortic and LV diastolic stiffness.⁵⁷ Other AGEs (ie, N-(carboxymethyl)lysine [CML]) do not cross-link proteins but may produce effects by interacting with receptors, leading to activation of a number of kinases and growth factors involved in inflammation, oxidative stress, and atherosclerosis.⁴⁻⁸

Thiazolium derivatives such as ALT-711 (3-phenacyl-4,5-dimethylthiazolium chloride; ALT) can break the protein cross-links formed by those AGEs that contain an α-dicarbonyl moiety.⁷⁹ Studies have reported improvements
in arterial stiffness indices with ALT therapy in diabetic or aged animals and in elderly humans. However, ALT also reduces systemic vascular resistance (SVR). Concomitant vasodilation complicates interpretation of the effects of ALT on indices of aortic stiffness because most are highly dependent on prevailing arterial tone. Furthermore, few studies have assessed the effect of ALT on arterial size. This is important because a therapy that alters collagen or elastin may promote arterial dilatation and further complicate assessment of arterial properties.

The objectives of this study were (1) to determine whether AGES were increased in elderly canines with experimental hypertension (old hypertensives [OH]) and (2) to examine the effect of chronic ALT therapy on vascular and ventricular function in this model. To avoid confounding effects of changes in resistance and/or artery dimension, we performed comprehensive assessment of arterial function using both pressure–dimension– and pressure–flow–based indices over changes in resistance and/or artery dimension, we performed comprehensive assessment of arterial function using both

**Methods**

All animal experiments were approved by the Mayo Institutional Animal Care and Use Committee. Euthanasia methods conformed to recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. The authors had full access to the data and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Elderly mongrel dogs (n=22, aged 8 to 12 years) underwent bilateral renal wrapping and placement of an abdominal aortic fluid-filled catheter for weekly blood pressure (BP) monitoring, as described previously. At 72 hours postoperatively, dogs were randomized to receive ALT-711 (1 mg/kg orally once daily) for 8 days or a control (no interventions) dogs (YN group) (n=11). At week 8, dogs underwent conscious echocardiography, invasive hemodynamic study under anesthesia, and tissue harvest. Previously harvested tissue from young (aged ~1 year), normal (no interventions) dogs (YN group) (n=6) was used as control for immunohistochemistry and Western blot analysis of CML.

**Echocardiography**

Two-dimensional guided M-mode echocardiography along with BP measurement was performed to assess LV volume (Teichholz formula), stroke volume, cardiac output, LV mass (American Society of Echocardiography criteria), and ejection fraction, as described previously. Effective arterial elastance (0.9×systolic BP/stroke volume), SVR (mean arterial pressure [MAP]×cardiac output), and systemic arterial compliance (SAC) (stroke volume/pulse pressure [PP]) were calculated.

**Acute Hemodynamic Study and Analysis**

Dogs were anesthetized with fentanyl (0.25 mg/kg bolus followed by 0.18 mg/kg per hour) and midazolam (0.75 mg/kg bolus followed by 0.59 mg/kg per hour), given autonomic blockade (propranolol 2 mg/kg and atropine 1 mg), and instrumented with LV and ascending aortic micromanometer catheters, piezoelectric crystals for LV volume and aortic dimensions, ascending aortic volumetric flow probe, atrial pacemaker, and inferior vena cava occluder. Dogs were atrial paced 10 to 20 bpm above sinus rate after autonomic blockade. Data were collected at baseline, after volume expansion with dextran (250 mL over 15 minutes), and during phenylephrine infusion to increase peripheral resistance with dose titrated to achieve systolic aortic pressure ~200 mm Hg.

At each data collection period, acute inferior vena cava occlusion was used to define the end-systolic pressure (ESP)–volume (ESV) relationship [ESP=Ee(Est−Vst)], where Ee is end-systolic elastance, and the end-diastolic pressure (EDP)–volume (EDV) relationship [EDP=e(alpha/D_O75)]. The time constant of LV relaxation (τ) was calculated as described previously.

MAP, cardiac output, and SVR were calculated from invasive aortic pressure and flow. Phasic aortic area change (Ao area Δ) was calculated as (π/4×D_A2−π/4×D_D2), where D_A and D_D are aortic systolic and diastolic dimensions. Aortic distensibility was calculated as (Ao area Δ/PP). Characteristic aortic impedance (Zv-volume; dyne·s·cm⁻³) was calculated similarly with the use of linear flow velocity (volumetric flow/aortic cross-sectional area). SAC was calculated by the method of Liu et al.

**Histological and Biochemical Analysis**

Total collagen and collagen solubility (pepsin digestion) were measured by the hydroxyproline assay, as described previously. Myocardial brain natriuretic peptide concentration was measured by radioimmunoassay to assess LV hypertrophy. Aortic wall thickness was measured in fixed specimen and averaged from 5 measurements.

**Immunohistochemistry**

Five-micrometer sections from canine LV or aorta were deparaffinized and rinsed with PBS. Three percent hydrogen peroxide in methanol was used for endogenous blocking. Antigen retrieval was performed by incubating slides with pepsin/HCl for 30 minutes at 37°C. Tissues were blocked for exogenous proteins with the use of normal rabbit serum 1:10 in PBS. Tissues were incubated in primary antibody CML:26 1:1000 for 60 minutes at room temperature in a humidity chamber. After they were rinsed with PBS, tissues were incubated with secondary rabbit anti-mouse biotin-F(ab)’2 for 30 minutes at room temperature (Jackson Immunoresearch, Westgrove, Pa) followed by incubation with ABCComplex/horseradish peroxidase for 1 hour. Visualization was achieved by a 5-minute diaminobenzidine (Sigma-Aldrich, St Louis, Mo) incubation and counterstained with Gill’s hematoxylin.

Aortic sections were scored by an investigator blinded to study group on a scale of 0 (no staining) to 4 (dense staining). In LV, staining was only seen in epicardial and occasional intramyocardial muscular arteries, and thus a score was not used. Sequential cuts of aortic and LV tissue were stained with picrosirius red and Lawson’s elastic van Gieson stains to illustrate CML distribution in relation to collagen or elastin, respectively.

**Western Analysis**

Five hundred milligrams of tissue was Polytron homogenized in 1 mL Triton lysis buffer (20 mmol/mL Tris-HCl, pH 7.5, 150 mmol/mL NaCl, 1 mmol/L EDTA, 1% Triton) plus 1 mL dithiothreitol and 4 mL of protease inhibitor cocktail (Calbiochem, Gibbstown, NJ). Protein concentration was determined by the Bradford method (Bio-Rad, Hercules, Calif), and 40 μg protein was loaded on a 10% SDS-PAGE gel and electrophoretically transferred to a 0.45-μm polyvinylidene fluoride membrane (Millipore, Bedford, Mass). The membrane was incubated in 2 μg/mL α-CML antibody (R&D Systems, Minneapolis, Minn), 2.5 μg/mL α-CML antibody (Cosmo Biotech, Tokyo, Japan), α-CML:26 1:1000, or α-GAPDH 1:5000 (Abcam, Cambridge, Mass) at 4°C overnight. Membranes were probed with secondary peroxidase-conjugated anti-F(ab)’2 fragment goat anti-mouse IgG (Jackson Immunoresearch, Westgrove, Pa) for 1 hour. Probed proteins were detected with the use of SuperSignal

Shapiro et al AGE in Elderly Hypertensives 1003
Chemiluminescent Substrate (Pierce, Rockford, Ill). Densitometry was completed with the use of Bio-Rad Chemidoc software.

Statistical Analysis

The Student t test was used to assess between-group comparisons. Simple and multiple linear regression was performed to assess associations between variables with log transformation of skewed variables as needed to satisfy modeling assumptions. Interactions between group and the other tested covariates were tested by introducing an interaction term. Two-way, repeated-measures ANOVA was used to compare variables between groups over time introducing an interaction term. Two-way, repeated-measures ANOVA was used to compare variables between groups over time.

Results

Evolution of BP After Renal Wrapping

ALT-treated dogs had lower systolic BP and PP after renal wrapping (Figure 1).

Conscious Assessment of LV and Vascular Function

Heart rate and LV end-diastolic volume were similar between groups (Table). Ejection fraction and stroke volume tended to be higher and systolic BP was lower in the OH+ALT dogs. Arterial elastance was lower in treated dogs, with reductions in both the fixed (lower SVR) and pulsatile (higher SAC) components of arterial load (Figure 2A to 2C). SAC was in both the fixed (lower SVR) and pulsatile (higher SAC) components of arterial load (Figure 2A to 2C). SAC was not different between groups (Figure 2D). However, there were few points with overlapping SVR between groups.

Invasive Assessment of Vascular Function

After anesthesia, instrumentation, and autonomic blockade, heart rate was lower in OH-ALT dogs (Table).

Pressure–Dimension–Based Indices of Aortic Stiffness

Systolic and diastolic aortic areas increased with increasing systolic and diastolic aortic pressure, respectively (P<0.001 for both) but were larger at any corresponding aortic pressure in the OH+ALT group. The slope of the relationship between aortic area and pressure was similar between groups (Figure 3A and 3B). Adjusting for dog weight or heart rate did not alter this finding. Aortic wall thickness was decreased in the OH+ALT group (Figure 3C). Phasic aortic area change was greater in ALT dogs (57±20 versus 39±13 mm²; P<0.0002), and this difference persisted after adjustment for MAP. Phasic aortic distensibility decreased with increasing MAP (P<0.001). The relationship between phasic aortic distensibility and MAP was shifted upward in ALT-treated dogs (but with similar slope), suggesting greater distensibility in ALT-treated dogs at any given distending pressure (Figure 3D).

Pressure–Flow–Based Indices of Arterial Properties

Zc-volume was independent of MAP (P>0.05) and was lower in OH+ALT dogs (Figure 4A). Zc-velocity was independent of MAP (P>0.05) and was similar between groups (P=0.25; Figure 4B). SAC was higher in OH+ALT dogs (Figure 4C), decreased with increasing MAP or SVR (P<0.001 for both), but was higher in OH+ALT dogs at any SVR (Figure 4D), indicating increased arterial compliance.

Table. Characteristics of Untreated and Treated Elderly Hypertensive Dogs

<table>
<thead>
<tr>
<th></th>
<th>OH</th>
<th>OH+ALT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>126±31</td>
<td>119±21</td>
<td>0.58</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>199±17</td>
<td>177±26</td>
<td>0.04</td>
</tr>
<tr>
<td>LV EDV, mL</td>
<td>95±18</td>
<td>100±20</td>
<td>0.56</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>56±12</td>
<td>63±9</td>
<td>0.18</td>
</tr>
<tr>
<td>Stroke volume</td>
<td>53±14</td>
<td>63±15</td>
<td>0.11</td>
</tr>
<tr>
<td>LV mass/body weight</td>
<td>5.84±1.12</td>
<td>4.96±1.02</td>
<td>0.07</td>
</tr>
<tr>
<td>Aortic structure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic collagen, μg/mg</td>
<td>68.2±19.3</td>
<td>56.2±10.4</td>
<td>0.10</td>
</tr>
<tr>
<td>Aortic collagen solubility, % insoluble</td>
<td>11.4±6.5</td>
<td>12.9±4.7</td>
<td>0.92</td>
</tr>
<tr>
<td>LV function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>102±13</td>
<td>88±6</td>
<td>0.001</td>
</tr>
<tr>
<td>EDV, mL</td>
<td>60±18</td>
<td>55±12</td>
<td>0.6</td>
</tr>
<tr>
<td>EDP, mm Hg</td>
<td>10±5</td>
<td>13±5</td>
<td>0.33</td>
</tr>
<tr>
<td>Es, mm Hg/mL</td>
<td>4.82±2.70</td>
<td>4.99±2.54</td>
<td>0.88</td>
</tr>
<tr>
<td>Vp, mL</td>
<td>4±15</td>
<td>4±9</td>
<td>0.98</td>
</tr>
<tr>
<td>β, mm Hg/mL</td>
<td>0.053±0.032</td>
<td>0.042±0.026</td>
<td>0.38</td>
</tr>
<tr>
<td>α</td>
<td>1.31±1.39</td>
<td>2.20±1.34</td>
<td>0.14</td>
</tr>
<tr>
<td>τ, ms</td>
<td>47±9</td>
<td>50±11</td>
<td>0.49</td>
</tr>
<tr>
<td>LV structure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV mass/body weight, g/kg</td>
<td>5.6±1.2</td>
<td>5.0±0.7</td>
<td>0.20</td>
</tr>
<tr>
<td>LV brain natriuretic peptide, pg/mg protein</td>
<td>0.42±0.38</td>
<td>0.19±0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>LV collagen, μg/mg tissue</td>
<td>2.6±0.7</td>
<td>2.9±0.7</td>
<td>0.33</td>
</tr>
<tr>
<td>LV collagen solubility, % insoluble</td>
<td>67.9±4.6</td>
<td>70.4±3.6</td>
<td>0.24</td>
</tr>
</tbody>
</table>
The slope of the relationship between SAC and SVR was similar between groups.

**Aortic Structure**

The total collagen in the OH+ALT aortas tended to be lower than in the OH group, but there was no difference in collagen solubility (Table).

There was negligible staining for CML in YN aorta (Figure 5A), but CML staining (α-CML2621 antibody shown here) was prominent in OH dogs (Figure 5B). Staining was not localized to areas of collagen (Figure 5C) or elastin (Figure 5D) but rather to smooth muscle and matrix proteins within the aortic wall and to the vasa vasorum, where CML staining was prominent in the vascular smooth muscle. The aortic immunohistochemistry score was increased similarly in OH and OH+ALT compared with YN dogs (Figure 5E). On Western analysis, CML content in aorta was increased similarly in OH and OH+ALT dogs compared with YN dogs (Figure 5F and 5G). The representative blot is shown with R&D Systems α-CML antibody, but findings were similar with the other antibodies.

**Invasive Assessment of Ventricular Function**

At invasive study and before volume expansion or phenylephrine infusion, LV EDV and EDP, Ees, V0, and S′ were all similar between groups (Table). By 2-way repeated-measures ANOVA assessing changes in these parameters over the experimental periods and between groups, there was no significant difference.
no difference in any of these variables between groups (group
\(P>0.05\) for all) or any difference in the way in which the
variables changed with volume expansion or phenylephrine
infusion (interaction \(P>0.05\) for all).

**Ventricular Structure**

LV mass/body weight ratio at echocardiography and autopsy
and LV brain natriuretic peptide all tended to be lower in the
OH+ALT group, suggesting less hypertrophy (Table). LV
collagen content and solubility were similar. By immunohis-
tochemistry, CML staining was not evident in the LV
myocardium or areas of collagen deposition in YN (Figure
6A and 6B) or OH (Figure 6C and 6D) dogs but was present
in some muscular arteries in YN (Figure 6A) and OH (Figure
6C) dogs. Findings in the OH+ALT group were similar (not
shown). Consistent with the immunohistochemistry findings,
CML was undetectable in all 3 groups on Western blot
analysis (regardless of antibody used).

**Discussion**

These data demonstrate the presence of AGEs as assessed by
CML content in an elderly hypertensive nondiabetic model
and indicate that AGE deposition, at least as assessed by
CML content, is confined to vascular smooth muscle cells in
the aorta, the aortic vasa vasorum, and the LV vessels and is
not apparent in areas of aortic collagen or elastin or LV
collagen. Accordingly, an AGE cross-link breaker had clear
effects on aortic dimension, phasic distensibility, stiffness,
and vascular tone but not on LV diastolic properties. These
data suggest that AGE accumulation and AGE protein cross-
linking in elderly nondiabetic hypertensives may influence
arterial properties via effects on proteins other than collagen
and elastin.

**CML Content and Localization in Elderly Hypertensive Dogs**

Similar to previous studies in human or experimental diabe-
tes, CML was localized to aortic smooth muscle\(^{22}\) and to the
vasa vasorum but did not appear to be associated with
collagen or elastin in the aorta. We cannot exclude the
presence of CML in vascular endothelial cells as well. CML
was essentially undetectable in the LV in the OH dogs by
both immunohistochemistry and Western analysis. Given the
dramatic effects of ALT on LV diastolic properties in a
previous study of elderly dogs,\(^{14}\) we confirmed the absence
of CML on Western analysis of LV tissue using 3 different
\(\alpha\)-CML antibodies. We observed some staining of arterial
smooth muscle in vessels in the LV, as described previously
in experimental or human diabetes,\(^{21,23}\) but this was evident in
some YN samples as well.

Although CML is commonly used as a marker of AGE
accumulation, the relationship of CML content to that of
other AGES and differences in tissue deposition of CML and
other AGES throughout the body are poorly defined.\(^{4}\) Because
CML does not form cross-links,\(^{4,7,22}\) this AGE may not
decrease with administration of a cross-link breaker as
observed here. CML formation increases with oxidative stress
but can increase with hypertension in the absence of increases
in oxidative stress.\(^{24}\) Some studies have shown a decrease in
CML with ALT therapy in diabetic models,\(^{22,25}\) an effect
postulated to be mediated by reduction in copper-catalyzed
glycoxidation.\(^{22}\) The lack of reduction in CML with ALT in
the present study may suggest that the effects of ALT in this
particular model are not mediated primarily by a reduction in
oxidative stress.

**Effect of ALT on Arterial Structure and Function**

ALT therapy attenuated development of hypertension and
increases in PP in the renal wrapping model. The effect of
ALT appeared apparent very early, consistent with in vitro studies with its precursor molecule that demonstrated a very rapid (within hours) breaking of protein cross-links.9 Whether the rapid effects were due to disruption of collagen cross-links or modification of other proteins is unclear given the aforementioned lack of CML deposition in collagen-rich areas of the aorta or aortic vasa vasorum. Measured in the conscious state, SAC was increased in the ALT group, consistent with studies in diabetic rats and elderly humans,11,12 but whether this effect was independent of the effect on vascular tone is difficult to say given the lack of overlap in SVR in the 2 groups in the conscious state.

Effects of ALT on systolic BP or PP in humans with essential or age-related systolic hypertension have been observed as well.12,26 SVR was lower in ALT-treated conscious dogs, a finding that has been observed with ALT therapy in elderly dogs and monkeys and in diabetic rats11,13,14 but not in elderly humans.12 ALT may reduce SVR via effects on endothelial function,27,28 although others have speculated that ALT may influence the biomechanical properties of the resistance vessels or the mechanical properties of the carotid sinus.11,29 The consistent presence of CML in vascular smooth muscle cells in the aorta, the aortic vasa vasorum, and the LV arteries noted here provides support for an effect of CML or other AGEs on vascular smooth muscle function that goes beyond extracellular matrix collagen cross-linking.

Data from the anesthetized study in which peripheral tone and output were varied over a large range in both groups reveal more information about the effect of ALT on aortic function. A highly significant effect of ALT to increase aortic dimension was observed. This occurred despite lower BP over the course of the model and was independent of distending pressure or body size. Although it was postulated that ALT could reduce the stiffness of the aorta, few studies have described the effect of ALT on vascular size. The careful study of Wolffenbuttel et al11 reported increases in carotid artery dimension in diabetic rats treated with ALT and, consistent with our findings, demonstrated that this was present in vivo and in vitro and was independent of distending pressure or aortic smooth muscle tone. Other studies of the effects of ALT on arterial properties did not assess effects on aortic size.10,12,13,30

Another major finding from the present study was that phasic aortic area change and phasic aortic distensibility were increased in ALT-treated dogs. Consistent with this finding,
pressure flow indices (Zc-volume, SAC, and the relationship between SAC and SVR) also suggested an improvement in aortic properties with ALT, as described previously in diabetic rats and elderly monkeys,\textsuperscript{11,13} although the findings with Zc-velocity (which controls for differences in vessel size) were less dramatic. Whereas age-related elastin degradation leads to aortic dilatation and increases in Zc and pulse wave velocity,\textsuperscript{1} here aortic dilatation was not associated with increased stiffness, a unique finding that underscores the fundamental difference in the mechanism of the aortic dilatation with ALT therapy.

These alterations in aortic function were associated with a trend toward decreases in aortic collagen and a decrease in aortic wall thickness, but aortic collagen solubility was not higher in OH+ALT dogs. Collagen solubility in tissue with very high collagen content (ie, rat tail skin) has been used as a marker of changes in AGE cross-links,\textsuperscript{4} and we did not collect such tissue for analysis. Although a trend toward reduction in total aortic collagen was observed and may suggest enhanced susceptibility to collagen degradation with ALT therapy,\textsuperscript{31} this was not statistically significant.

**Effect of ALT on LV Structure and Function**

There was a trend toward less hypertrophy in the ALT-treated dogs observed with all indices used to assess hypertrophy (echo LV mass, autopsy weights, and LV brain natriuretic peptide concentration), but none reached statistical significance. We speculate that ALT reduced hypertrophy but that small numbers, unpaired study design, and the modest magnitude of the reduction\textsuperscript{30} hindered our ability to unequivocally demonstrate it. Indeed, Little et al\textsuperscript{30} found a significant decrease in LV mass of \(\approx 4\%\) in paired analysis (pretreatment to posttreatment) with ALT treatment in humans with diastolic heart failure.

In contrast to a previous study in elderly dogs,\textsuperscript{14} we did not observe a difference in LV EDV or LV diastolic properties between the 2 groups. This may be due to the difference in the experimental model (aged dogs versus aged dogs with experimental hypertension) or to the marked differences in the methods used to assess LV diastolic properties. In the present study, pressure and volume were simultaneously and instantaneously assessed, the pericardium was open, and heart rate, respiration, and autonomic tone were controlled. Acute preload reduction was used to define the entire curvilinear end-diastolic pressure–volume relationship, and this was repeated over a range of preloads and afterloads. The study of Asif et al\textsuperscript{14} used echocardiography in conscious dogs with pericardium intact and collected only 2 pressure and volume data points, before and after marked acute volume expansion without autonomic blockade, heart rate, or respiratory effort control or reduction in preload to define the end-diastolic pressure–volume relationship independent of extrinsic forces. The effect on diastolic properties in the previous study was dramatic and is difficult to reconcile with our findings. The more dramatic effects in the previous study could also reflect the paired study design. The dose and duration of therapy were similar to those used here. Nonetheless, the difference in vascular and LV effects with ALT in this model is consistent with the differences in AGE accumulation as assessed by CML content. We speculate that effects on LV properties may be more dramatic in the presence of diabetes or renal dysfunction, in which case LV AGE content\textsuperscript{4,25} and sequelae\textsuperscript{32} may be more dramatic. However, it is of note that a recent study found CML deposition confined to the LV vessels in diabetic humans with heart failure.\textsuperscript{23}

**Limitations**

We did not assess the presence of other AGE structures or changes in the type of collagen with ALT therapy. A single dose and duration of therapy were used. Investigators were not blinded to treatment group. Because BP was not measured until 1 week after surgery, we cannot exclude baseline differences in BP between groups, but assignment was random, and the findings were consistent with previous studies and the presence of CML in the aorta. Because of the relatively small sample size and multiple comparisons,
the chance of a false-positive or -negative result is acknowledged.

Conclusions
AGEs as assessed by CML content were increased in the aorta but not in the LV in an elderly hypertensive nondiabetic model, were localized to vascular smooth muscle cells, and were not associated with areas of collagen deposition in the aorta or LV. Therapy with an AGE cross-link breaker had clear effects on aortic stiffness and on vascular tone but was associated with significant aortic dilatation and a lack of effect on LV diastolic properties. On the basis of these data, we speculate that AGE accumulation and AGE protein cross-linking in elderly hypertensive subjects without diabetes may influence arterial properties via effects on proteins other than collagen and elastin. Furthermore, the potential for AGE-modifying therapies to improve vascular or ventricular properties deserves further study but should include careful attention to potential changes in vascular size.

Sources of Funding
Drug and partial financial support for the study was provided by Alteon, Inc. Drs Redfield (HL 63281 and HL 76611), Mohammed (HL 76611), and Owan (HL 07111) were funded in part by the National Institutes of Health.

Disclosures
Dr Redfield received research funding from Alteon, Inc. The other authors report no potential conflicts of interest.

References
CLINICAL PERSPECTIVE

Advanced glycation end products (AGEs) are believed to increase left ventricular and vascular stiffness, in part via cross-linking proteins. We determined whether and where AGEs were increased in elderly hypertensive nondiabetic dogs and whether an AGE cross-link breaker (ALT-711) improved vascular or ventricular function. N-(Carboxymethyl)lysine, a marker of AGE accumulation, was markedly increased in aortic and aortic vasa vasorum smooth muscle but was not localized to areas of collagen or elastin and was not present in left ventricular myocardium of old hypertensive dogs. ALT-711 therapy was associated with lower blood pressure and pulse pressure, decreased systemic vascular resistance, increased aortic distensibility and arterial compliance, and, notably, significant aortic dilatation. Neither left ventricular systolic nor diastolic function was different in ALT-711–treated dogs. AGE-modifying therapies have been and continue to be tested in patients with heart failure and hypertension. These data in a nondiabetic model with clinical relevance to human hypertension and heart failure suggest that this therapeutic strategy may favorably modify arterial properties but also that such therapies may be associated with aortic dilatation, at least in the setting of persistent hypertension. Thus, aortic size should be monitored carefully in clinical trials of these agents. Furthermore, at least in this nondiabetic model, we did not find evidence of a favorable effect on diastolic function. Whether more prolonged therapy or therapy in diabetics with heart failure or hypertension would be associated with benefit on diastolic function remains to be established.
Advanced Glycation End Products Accumulate in Vascular Smooth Muscle and Modify Vascular but Not Ventricular Properties in Elderly Hypertensive Canines
Brian P. Shapiro, Theophilus E. Owan, Selma F. Mohammed, Donna M. Meyer, Lisa D. Mills, Casper G. Schalkwijk and Margaret M. Redfield

Circulation. 2008;118:1002-1010; originally published online August 18, 2008; doi: 10.1161/CIRCULATIONAHA.108.777326
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/118/10/1002

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 is online at:
http://circ.ahajournals.org/subscriptions/