



Advanced glycation end-products, anti-hypertensive treatment and diastolic function in patients with hypertension and diastolic dysfunction

Jasper W.L. Hartog^{1*}, Ruud M. van de Wal⁵, Casper G. Schalkwijk³, Toshio Miyata⁴, Wybren Jaarsma⁵, H.W. Thijs Plokker⁵, Leen M. van Wijk⁶, Andries J. Smit², Dirk J. van Veldhuisen¹, and Adriaan A. Voors¹

¹Department of Cardiology, University Medical Center Groningen and University of Groningen, Hanzeplein 1, PO Box 30001, 9700 RB Groningen, The Netherlands; ²Department of Medicine, University Medical Center Groningen and University of Groningen, Hanzeplein 1, PO Box 30001, 9700 RB Groningen, The Netherlands; ³Department of Medicine, Academic Hospital Maastricht, Debyeilaan 25, PO Box 5800, 6202 AZ Maastricht, The Netherlands; ⁴Center for Translational and Advanced Research, Tohoku University Graduate School of Medicine, Miyagi, Japan; ⁵Department of Cardiology, St Antonius Hospital, Nieuwegein, The Netherlands; and ⁶Department of Cardiology, Refaja Hospital, Stadskanaal, The Netherlands

Received 17 June 2009; revised 6 November 2009; accepted 25 November 2009

| | |
|----------------------------|---|
| Aims | To investigate the relationship between advanced glycation end-products (AGEs) and diastolic function and the response to blood pressure treatment in patients with hypertension and diastolic dysfunction. |
| Methods and results | Data were analysed from 97 patients (aged 65 ± 10 years, 36% male) who were randomly assigned to 6 months open-label treatment with either eprosartan on top of other anti-hypertensive drugs ($n = 47$) or other anti-hypertensive drugs alone ($n = 50$). Tissue AGE accumulation was measured using a validated skin-autofluorescence (skin-AF) reader ($n = 26$). Plasma N^{ϵ} -(carboxymethyl)lysine (CML), N^{ϵ} -(carboxyethyl)lysine (CEL), and pentosidine were measured by LC-MS/MS and HPLC. Diastolic function was assessed using echocardiography. Blood pressure was reduced from 157/91 to 145/84 mmHg ($P < 0.001$) in the eprosartan group and from 158/91 to 141/83 mmHg ($P < 0.001$) in the control group. No effect of eprosartan was found on AGE levels. In patients with baseline skin-AF $<$ median, E/A ratio ($P = 0.04$) and the mean peak early-diastolic filling velocity (E') improved ($P = 0.001$). In contrast, in patients with skin-AF levels $>$ median, E/A ratio ($P = 0.84$) and mean E' ($P = 0.32$) remained unchanged. |
| Conclusion | Although eprosartan did not decrease levels of AGEs, patients with lower skin-AF at baseline showed a larger improvement in diastolic function in response to either anti-hypertensive treatment compared with patients with higher skin-AF. |
| Keywords | Hypertension • Advanced glycation end-products • Diastolic function • Skin-autofluorescence • Tissue velocity imaging |

Introduction

Hypertension is related to a higher risk for development of heart failure, particularly diastolic heart failure. Several mechanisms underlying diastolic heart failure have been proposed.^{1–5} Besides the influence of extra cardiac factors like preload, and afterload,

several intra cardiac factors exist that have been shown to influence diastolic function. On a functional level, residual diastolic cross-bridge interaction may generate diastolic force and therefore determine diastolic function. Structurally, both cytoskeletal components as well as matrix components have been shown to influence diastolic tension, especially at high sarcomere length.

* Corresponding author. Tel: +31 50 361 2355, Fax: +31 50 361 4391, Email: j.w.l.hartog@thorax.umcg.nl

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2010. For permissions please email: journals.permissions@oxfordjournals.org.

The latter components may be subjected to modifications by the formation of advanced glycation end-products (AGEs). These are carbohydrate and lipid-dependent modifications of protein, formed by oxidative and non-oxidative reactions.⁶ Advanced glycation end-product formation affects the physiological properties of proteins in the extracellular matrix, such as turnover, and elasticity.

Recently, we demonstrated that skin-AF, a measure of tissue AGEs is strongly related to diastolic function in dialysis patients.⁷ It remains unknown whether AGEs are related to diastolic function in patients with hypertension and signs of diastolic dysfunction. *In vitro* and *in vivo* studies have shown that angiotensin II type 1 receptor blockers (ARBs) can reduce AGE formation.^{8–10} Angiotensin II type-1 receptor blockers prevent the production of reactive carbonyl and dicarbonyl compounds (RCOs), which are critical precursors of AGEs.^{8–10} However, conflicting clinical data on the effects of ARBs on AGE accumulation have been presented.^{9–12}

Therefore, the first aim of this study was to evaluate the effects of the ARB eprosartan vs. control anti-hypertensive treatment on both serum and tissue AGEs in a randomized clinical study in patients with hypertension and diastolic dysfunction. Secondly, we aimed to establish the influence of baseline tissue and serum AGEs on changes in diastolic function in response to eprosartan and control anti-hypertensive treatment.

Methods

Patients and study design

For the present analysis, we studied 97 patients, participating in a prospective randomized open-label multicentre trial with blinded end-point (PROBE design) that aimed to establish the effects of the angiotensin II type 1 receptor blocker (ARB) eprosartan in hypertensive patients with signs of diastolic dysfunction. The study was performed in three centres in the Netherlands. Patients older than 18 years, with hypertension (blood pressure repeatedly $\geq 140/90$ mmHg), who were not yet treated with an ARB, were screened for signs of diastolic dysfunction on echocardiography. Patients were eligible to participate if their left-ventricular ejection fraction (LVEF) was $\geq 50\%$, and E/A was < 1 in combination with either a deceleration time (Dct) > 280 ms or an isovolumetric relaxation time (IVRT) > 105 ms. Exclusion criteria were recent myocardial infarction (< 6 weeks), unstable angina pectoris, severe valvular disease, acute heart failure, atrial fibrillation, pacemaker, history of drug-sensitivity or allergy for eprosartan, pregnancy or lactation, clinically significant liver or renal disease, infection, or previous poor-quality echocardiogram. Five patients were permitted study entry, although they were already using an ARB. Patients were randomized to either eprosartan 600 mg once daily (400 mg for 2 weeks as loading dose) on top of other anti-hypertensive drugs ($n = 47$) or other anti-hypertensive drugs alone ($n = 50$) for a period of 6 months. Other anti-hypertensive drugs that were allowed included ACE-inhibitors, β -blockers, diuretics, and calcium antagonists. Baseline ($t = 0$ months) and follow-up ($t = 6$ months) measurements included echocardiography, skin-autofluorescence (skin-AF), circulating AGEs, and basic laboratory values. Estimated GFR (eGFR) was calculated using the sMDRD formula as described by Smilde et al.¹³ The severity of heart failure was determined in accordance with the NYHA functional class. This study complies with the Declaration of Helsinki. The study protocol was approved by the appropriate institutional review committee and all patients gave written informed consent.

Advanced glycation end-product accumulation measured by skin-autofluorescence

Tissue AGE accumulation was assessed using a validated skin-autofluorescence (skin-AF) reader as described previously.^{14,15} In short, the AGE-reader illuminates a skin surface of ~ 4 cm², guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation ~ 370 nm). Light from the skin is measured with a spectrometer in the 300–600 nm range, using 200 μ m glass fibre. As a measure of skin-AF the ratio between emission and excitation was calculated in arbitrary units (a.u.) by dividing the area under the curve between 420 and 600 nm by the area under the curve between 300 and 420 nm, and multiplying by 100. Skin-AF was measured at the volar side of the lower arm at ~ 10 –15 cm below the elbow fold. Care was taken to perform the measurement at normal skin site, i.e. without visible vessels, scars, lichenification, or other skin abnormalities. Intraobserver variation of repeated AFR measurements on 1 day was 6%. Skin-AF data was obtained in 26 patients, 8 of which were from the eprosartan group. Although measurements were performed by the study physician who was aware of the allocated treatment, the measurement technique does not allow manipulation of the results. Also, upon the availability of the AGE-reader technique all patients that were randomized underwent the AGE-reader measurement, thus preventing any selection (AGE-Reader; patent PCT/NL99/00607; DiagnOptics BV, Groningen, The Netherlands).

Determination of carboxymethyllysine and carboxyethyllysine levels

CML and CEL were determined by stable-isotope-dilution tandem mass spectrometry (LC-MS/MS) as described previously.¹⁶ In short, CML and CEL were liberated from plasma proteins by acid hydrolysis after addition of deuterated CML and CEL as internal standards. Chromatographic separation was performed by gradient-elution reversed-phase chromatography with a mobile phase containing 5 μ mol/L nonafluoropentanoic acid as ion-pairing agent. Mass transitions of 205.1 \rightarrow 384.1 and 219.1 \rightarrow 384.1 for CML and CEL, respectively, and 209.1 \rightarrow 388.1 and 223.1 \rightarrow 388.1 for their, respectively, internal standards were monitored in positive-ion mode. CML and CEL were separated by baseline resolution with a total analysis time of 21 min. Within-day and between-day coefficients of variation were < 4.4 and $< 3.2\%$ for CML, and < 6.8 and $< 7.3\%$ for CEL. CML and CEL analyses were performed by laboratory personnel who were unaware of the allocated treatment.

Determination of pentosidine levels

Pentosidine levels were measured by high performance liquid chromatography (HPLC) as described previously by Izuhara et al.¹⁷ Briefly, a 50 μ L solution of acid hydrolysate of plasma was injected into an HPLC system and separated on a C18 reverse-phase column (Waters, Tokyo, Japan). The effluent was monitored using a fluorescence detector (RF-10A; Shimadzu, Kyoto, Japan) at an excitation-emission wavelength of 335/385 nm. Synthetic pentosidine was used to obtain a standard curve. The limit of detection was 5 pmol of pentosidine per millilitre of plasma. Normal values in four healthy subjects averaged 0.114 ± 0.011 μ mol/L, with a coefficient of variation of $5.48 \pm 0.81\%$ on four different days. Pentosidine analyses were performed by laboratory personnel who were unaware of the allocated treatment.

Echocardiography

Patients underwent two-dimensional echocardiography, including colour flow mapping 2D-guided M-mode, blood pool, and tissue Doppler echocardiography. Echocardiography was performed by experienced cardiac technicians using a General Electric VIVID 7 system with a 2.5 MHz probe. Technicians were blinded for the allocated treatment. Measurements included left-ventricular and atrial dimensions, the peak early (E) and late (A) diastolic filling velocities, IVRT, deceleration time (slope) of the early peak filling (DCT). Furthermore, using tissue Doppler, early-diastolic velocity (E') was measured on the lateral, septal, anterior, and inferior wall areas, and subsequently averaged (mean E'). E/E' was calculated by dividing the peak early-diastolic filling (E) by the average E' measured using tissue Doppler. E' can be measured both by pulsed wave tissue Doppler (PW-TDI), which was used in two of the three centres ($n = 44$) and colour coded tissue Doppler (CC-TDI), which was used in one

centre ($n = 53$). Although both techniques are based upon the same principles and correlation is high ($R = 0.90$, $P < 0.001$), E' measurements obtained with CC-TDI are systematically lower than E' values obtained using PW-TDI. Based upon 105 subjects who were referred to our echocardiography department for evaluation of diastolic left-ventricular function, we calculated that the relation between PW-TDI and CC-TDI could be described by the following equation: $E' \text{ (PW-TDI)} = 1.2 \times E' \text{ (CC-TDI)} + 1.6$. Using this equation CC-TDI measurements were converted to PW-TDI measurements for further analyses.

Statistical analyses

Data were analysed using SPSS version 12.01 (SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as mean \pm SD or as median [25–75% interquartile range], where applicable. Nominal variables are expressed as n (%). Baseline characteristics were analysed for

Table 1 Baseline characteristics

| Variable | Total $n = 97$ | Eprosartan $n = 47$ | Standard $n = 50$ | P-value |
|---|-------------------|------------------------|----------------------|---------|
| Age (years) | 65 ± 10 | 65 ± 10 | 64 ± 10 | 0.52 |
| Male, n (%) | 35 (36) | 16 (34) | 19 (38) | 0.56 |
| NYHA functional class | 1.3 ± 0.5 | 1.4 ± 0.5 | 1.3 ± 0.5 | 0.17 |
| Diabetes mellitus, n (%) | 11 (11) | 5 (11) | 6 (12) | 0.51 |
| Physical examination | | | | |
| Systolic blood pressure (mmHg) | 158 ± 17 | 157 ± 16 | 158 ± 17 | 0.94 |
| Diastolic blood pressure (mmHg) | 91 ± 10 | 91 ± 10 | 91 ± 10 | 0.98 |
| Heart rate (b.p.m.) | 69 ± 12 | 70 ± 13 | 68 ± 12 | 0.37 |
| Body mass index (kg/m^2) | 29 ± 5 | 29 ± 4 | 29.2 ± 5.3 | 0.63 |
| AGE accumulation | | | | |
| Skin-AF (a.u.) | 2.4 ± 0.4 | 2.4 ± 0.4 | 2.4 ± 0.4 | 0.83 |
| CML ($\mu\text{mol/L}$) | 1.5 ± 0.3 | 1.40 ± 0.25 | 1.48 ± 0.37 | 0.54 |
| CEL ($\mu\text{mol/L}$) | 1.5 ± 0.4 | 1.52 ± 0.39 | 1.44 ± 0.36 | 0.35 |
| Pentosidine ($\mu\text{mol/L}$) | 0.16 ± 0.09 | 0.16 ± 0.11 | 0.15 ± 0.05 | 0.33 |
| Laboratory assessments | | | | |
| Haemoglobin (mmol/L) | 8.9 ± 0.7 | 8.9 ± 0.7 | 8.8 ± 0.7 | 0.35 |
| Creatinine (mmol/L) | 82 ± 21 | 84 ± 21 | 80 ± 21 | 0.29 |
| eGFR with MDRD (mL/min/1.73 m^2) | 76 ± 16 | 74 ± 16 | 79 ± 17 | 0.14 |
| Echocardiography | | | | |
| LVEF (%) | 60 [55–60] | 60 [60–60] | 60 [60–60] | 0.89 |
| E/A ratio | 0.82 [0.7–0.9] | 0.83 [0.7–0.9] | 0.81 [0.7–0.8] | 0.89 |
| Dct (ms) | 243 ± 55 | 239 ± 48 | 246 ± 60 | 0.54 |
| IVRT (ms) | 110 [90–140] | 110 [90–130] | 110 [90–140] | 0.29 |
| Average E' (cm/s) | 8.2 ± 2.2 | 8.0 ± 2.3 | 8.4 ± 2.1 | 0.39 |
| E/E' | 9.0 ± 3.3 | 9.3 ± 4.0 | 8.7 ± 2.5 | 0.35 |
| Medication, n (%) | | | | |
| ACE-inhibitors | 43 (44) | 19 (40) | 24 (48) | 0.45 |
| All receptor blockers | 5 (5) | 3 (6) | 2 (4) | 0.55 |
| β -blockers | 35 (36) | 17 (36) | 18 (36) | 0.99 |
| Ca-antagonists | 18 (19) | 9 (19) | 9 (18) | 0.62 |
| Diuretics | 30 (31) | 12 (26) | 18 (36) | 0.27 |

NYHA, New York Heart Association functional class; Skin-AF, skin autofluorescence; CML, carboxymethyllysine; CEL, carboxyethyllysine; eGFR, estimated glomerular filtration rate; LVEF, left-ventricular ejection fraction; E/A ratio, ratio between the peak early (E) and late (A) diastolic filling velocities; E/E' , ratio between the peak early-diastolic filling (E) and the average early-diastolic tissue velocity (E'); E' , early-diastolic tissue velocity; DCT, deceleration time; IVRT, isovolumetric relaxation time; ACE, angiotensin converting enzyme.

difference by treatment group using Student's *t*-test or Mann–Whitney *U* test where applicable for continuous variables and by χ^2 for nominal variables. The effects of eprosartan on AGEs, diastolic function, and blood pressure were evaluated using Student's *t*-test or Mann–Whitney *U* test where applicable. Analyses were done based upon the intention-to-treat principle. The overall differences between baseline and follow-up values for blood pressure, AGEs, and diastolic function were analysed using paired *t*-test as well as Wilcoxon signed ranks test as appropriate. Multivariable linear regression analysis was used to correct our results for possible confounders. The correlation between

AGEs and diastolic function was assessed using Pearson and Spearman's rho correlation where applicable. A $P \leq 0.05$ (two-sided) was considered statistically significant.

Results

Baseline characteristics are shown in Tables 1 and 2. Data were analysed from 97 patients (35 male) aged 65 ± 10 years who were randomly assigned to 6 months open-label treatment with either eprosartan and other anti-hypertensive drugs ($n = 47$) or other anti-hypertensive drugs alone ($n = 50$). The majority of patients (69%) were classified as NYHA functional class I, 29% were classified as NYHA functional class II, and 2% as NYHA functional class III. Only a few patients had diabetes mellitus (11%). Baseline systolic and diastolic blood pressures in the whole group were 158 ± 17 and 91 ± 10 mmHg, respectively. Mean skin-AF was 2.4 ± 0.4 a.u., which is markedly higher ($P < 0.001$) than in a normal control group (2.1 ± 0.5 a.u.) previously described by our group.¹⁸ CEL levels (1.5 ± 0.4 vs. 0.8 ± 0.2 $\mu\text{mol/L}$; $P < 0.001$) were higher and CML (1.5 ± 0.3 vs. 2.8 ± 0.4 $\mu\text{mol/L}$; $P < 0.001$) levels were lower than in a normal control group.¹⁶ Pentosidine (0.16 ± 0.09 vs. 0.11 ± 0.01 $\mu\text{mol/L}$; $P = 0.27$) levels were not significantly different from control patients.¹⁷ There were no significant differences in baseline characteristics between the two treatment groups. Furthermore, Table 2 shows that there were no significant differences in baseline characteristics between the patients that underwent skin-AF measurements and the other patients. At the end of study eprosartan

Table 2 Baseline characteristics of subgroup with skin-autofluorescence measurement

| Variable | Other patients <i>n</i> = 71 | Subgroup skin-AF <i>n</i> = 26 | <i>P</i> -value |
|---------------------------------------|---------------------------------|-----------------------------------|-----------------|
| Age (years) | 65 \pm 10 | 62 \pm 10 | 0.07 |
| Gender (male; <i>n</i> , %) | 24 (34) | 11 (42) | 0.64 |
| Diabetes mellitus (yes; <i>n</i> , %) | 10 (14) | 1 (4) | 0.16 |
| Systolic blood pressure (mmHg) | 157 \pm 16 | 158 \pm 20 | 0.87 |
| Diastolic blood pressure (mmHg) | 90 \pm 11 | 92 \pm 8 | 0.52 |
| Average <i>E'</i> (cm/s) | 8.0 \pm 2.3 | 8.6 \pm 1.5 | 0.24 |
| <i>E/E'</i> | 9.3 \pm 3.6 | 8.2 \pm 2.2 | 0.14 |

EE', ratio between the peak early-diastolic filling (*E*) and the average early-diastolic tissue velocity (*E'*); *E'*, early-diastolic tissue velocity.

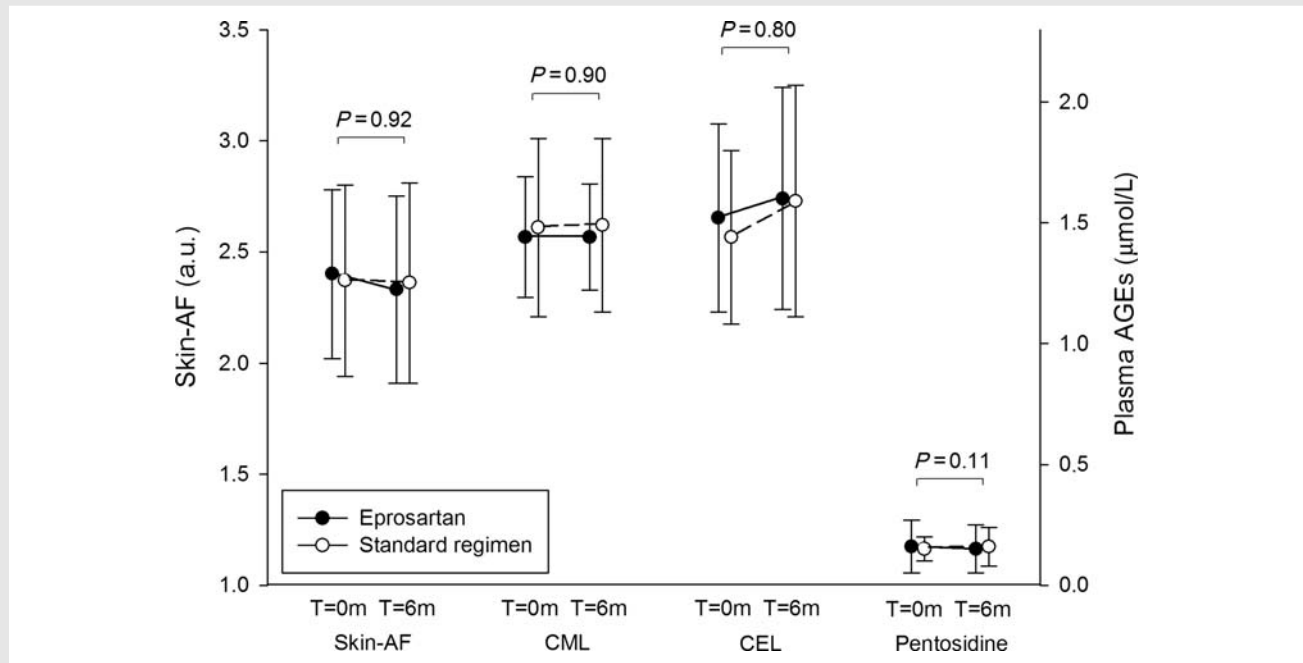


Figure 1 Changes in advanced glycation end-product accumulation stratified by treatment group. The figure depicts changes in AGE accumulation stratified for treatment group. *P*-values denote the differences in the change in AGE accumulation between the treatment groups calculated with Student's *t*-test or Mann–Whitney *U* test where applicable. AGEs, advanced glycation end-products; Skin-AF, skin-autofluorescence; CML, carboxymethyllysine; CEL, carboxyethyllysine. Values are means with standard deviations. Skin-AF, CML, CEL, and pentosidine data were available in 26, 96, 96, and 96 patients, respectively.

Table 3 Correlations of baseline advanced glycation end-product levels with diastolic function at baseline, 6 months, and the delta between baseline and 6 months

| Diastolic function | Correlation coefficients (R) of baseline AGEs with diastolic function | | | |
|--------------------|---|--------|--------|--------|
| | Skin-AF | CML | CEL | Pent |
| Baseline | | | | |
| E/A ratio | −0.08 | 0.13 | 0.08 | −0.03 |
| IVRT | 0.14 | −0.16 | −0.12 | −0.03 |
| DCt | −0.04 | −0.13 | 0.10 | 0.00 |
| E' | −0.17 | 0.06 | −0.10 | −0.13 |
| EE' | −0.12 | −0.12 | 0.00 | −0.03 |
| Delta | | | | |
| E/A ratio | −0.41* | −0.02 | −0.06 | 0.12 |
| IVRT | −0.16 | 0.16 | −0.06 | −0.00 |
| DCt | 0.12 | −0.102 | −0.011 | −0.15 |
| E' | −0.46** | 0.08 | −0.07 | 0.10 |
| EE' | 0.09 | −0.05 | −0.04 | −0.07 |
| 6 months | | | | |
| E/A ratio | −0.43* | 0.07 | −0.00 | 0.05 |
| IVRT | −0.13 | 0.11 | −0.15 | −0.02 |
| DCt | 0.22 | −0.13 | 0.07 | −0.24* |
| E' | −0.57** | 0.16 | 0.01 | 0.03 |
| EE' | −0.09 | −0.20 | −0.09 | −0.14 |

R, correlation coefficient; Skin-AF, skin autofluorescence; CML, carboxymethyllysine; CEL, carboxyethyllysine; Pent, Pentosidine; EE', ratio between the peak early-diastolic filling (E) and the average early-diastolic tissue velocity (E'); E', early-diastolic tissue velocity.

* = $P < 0.05$.

** = $P < 0.01$.

was stopped in 7/47 patients. Blood pressure was reduced from 157/91 to 145/84 mmHg ($P < 0.001$) in the eprosartan group and from 158/91 to 141/83 mmHg ($P < 0.001$) in the control group. Changes in systolic (-13 ± 19 vs. -16 ± 17 mmHg; $P = 0.74$) and diastolic blood pressure (-7 ± 10 vs. -7 ± 10 mmHg; $P = 0.38$) were not significantly different between the eprosartan and control group. Furthermore, changes in blood pressure were not different between patients with AGE levels above the median when compared with patients with levels below the median (data not shown).

The effects of eprosartan on AGE accumulation are depicted in Figure 1. Eprosartan use had no significant effects on changes in skin-AF (-0.06 ± 0.3 vs. -0.07 ± 0.3 a.u.; $P = 0.92$), CML (-0.06 ± 0.5 vs. 0.01 ± 0.1 $\mu\text{mol/L}$; $P = 0.90$), CEL (0.01 ± 0.7 vs. 0.05 ± 0.6 $\mu\text{mol/L}$; $P = 0.80$), or pentosidine (-0.025 ± 0.1 vs. 0.008 ± 0.1 $\mu\text{mol/L}$; $P = 0.11$). Thus, overall, no effects of eprosartan on either plasma or tissue AGEs were found. In the whole group, CEL levels showed a small increase (1.47 ± 0.39 vs. 1.58 ± 0.46 ; $P = 0.01$), while skin-AF and levels of CML, and pentosidine remained unchanged ($P = 0.27$; $P = 0.62$; $P = 0.62$, respectively). Despite a large reduction in blood pressure, no effects of either eprosartan or control anti-hypertensive therapy on diastolic function were found (data not shown).

The second aim of the study was to relate baseline plasma and tissue AGEs to changes in diastolic function in response to blood pressure therapy. Table 3 depicts the relationship between baseline AGE levels and (changes in) diastolic dysfunction. The level of baseline plasma AGEs was not related to (changes in) diastolic function. Also, baseline tissue AGEs were unrelated to baseline diastolic function. In contrast however, baseline tissue AGEs were related to response to therapy. Table 3 shows that lower skin-AF at baseline was related to an improvement in E/A ratio ($P = 0.023$) and an improvement in mean tissue E' ($P = 0.01$).

Figure 2 shows a graphic representation of the relationship between baseline skin-AF ($>$ median vs. $<$ median) and diastolic function at baseline and after 6 months of therapy. In patients with baseline skin-AF $<$ median, E/A ratio [from 0.84 (0.69–0.92) to 0.92 (0.85–1.04), $P = 0.04$] and E' (from 8.7 ± 1.7 to 10.2 ± 1.5 cm/s, $P = 0.001$) improved compared with patients with skin-AF levels $>$ median in whom E/A ratio [from 0.85 (0.71–0.99) to 0.84 (0.74–0.98), $P = 0.84$] and E' (from 8.4 ± 1.4 to 8.1 ± 1.3 cm/s, $P = 0.32$) remained unchanged. There was an indication of an improvement of EE' in patients with skin-AF levels $<$ median (from 8.5 ± 1.8 to 8.1 ± 1.4 cm/s, $P = 0.36$) compared with patients with skin-AF levels $>$ median (from 7.7 ± 2.4 to 7.8 ± 1.6 cm/s, $P = 0.98$). No significant differences between patients with skin-AF $<$ median compared with skin-AF $>$ median were observed for DCT ($P = 0.71$ vs. $P = 0.61$, respectively), and IVRT ($P = 0.48$ vs. $P = 0.32$, respectively). Using multi-variable linear regression analysis we further validated our results by correcting for possible confounders. The significant relations that were found for skin-AF with change in E' and change in E/A ratio persisted after correction for age, renal function (eGFR), and the presence of diabetes.

Discussion

The results of the current study indicate that neither the ARB eprosartan nor control anti-hypertensive treatment decreased levels of plasma and tissue AGEs in patients with hypertension and diastolic dysfunction. Interestingly, however, we showed that in patients with lower skin-AF at baseline, diastolic function improved, in contrast to a lack of improvement in those with a skin-AF level above the median.

Angiotensin II type 1 receptor blockers have shown the ability to lower *in vitro* and *in vivo* AGE formation and are thought to do so mainly by preventing the production of RCOs, which are critical precursors of AGEs.^{8–10} However, conflicting data from clinical studies on the effects of ARBs on AGE accumulation have been presented. While two smaller studies by Saisho *et al.*⁹ and Monacelli *et al.*¹⁰ found that ARBs lowered plasma AGEs, two larger trials showed no effects of ARBs on AGE accumulation.^{11,12} One reason may be that ARBs at the doses used in the clinical situation (significantly lower than that for the *in vitro* studies) do not provide sufficient inhibition of plasma and tissue AGE formation. However, in the current study a possible treatment effect of ARBs may have been overshadowed by the fact that 44% of our patients were using an ACE-inhibitor at study entry.

Patients with lower tissue skin-AF at baseline showed a significant improvement in diastolic function in response to

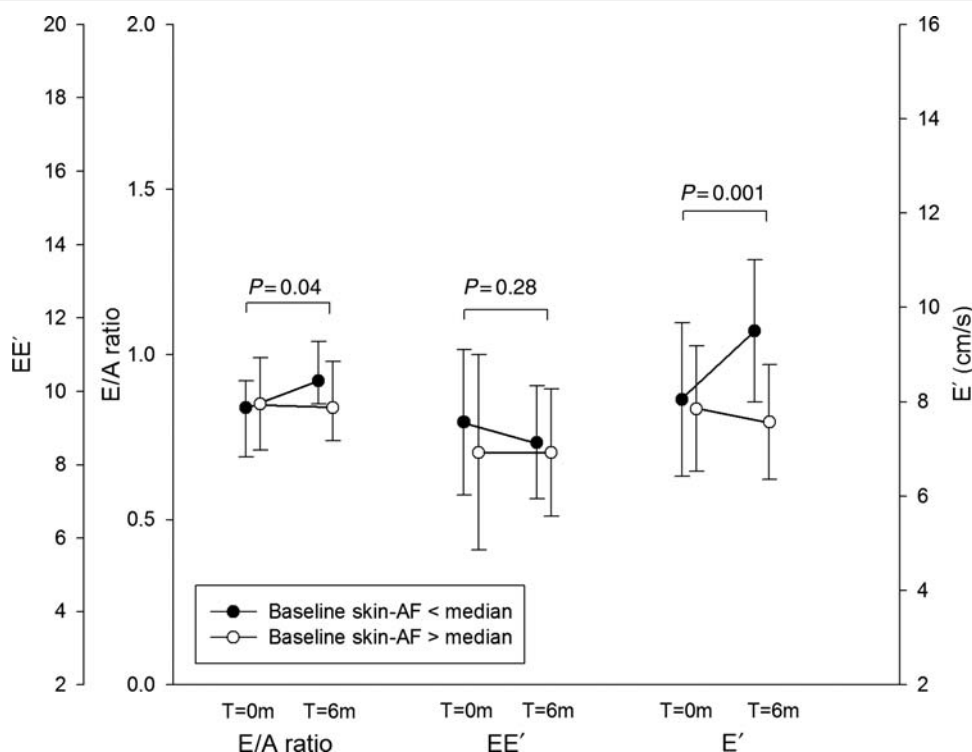


Figure 2 Changes in diastolic function stratified for baseline skin-autofluorescence. The figure depicts changes in diastolic function stratified for baseline skin-autofluorescence. *P*-values denote the differences of the changes in diastolic function in the skin-autofluorescence group < median calculated with a paired Student's *t*-test or a Wilcoxon signed ranks test as appropriate. E/A ratio, ratio between the peak early (E) and late (A) diastolic filling velocities; EE', ratio between the peak early-diastolic filling (E) and the average early-diastolic tissue velocity (E'); E', early-diastolic tissue velocity. Depicted are mean values with standard deviations (E' and EE') and median values with 25–75% interquartile range (E/A ratio) as appropriate. Skin-autofluorescence data was available in 26 patients.

anti-hypertensive therapy, while patients with higher skin-AF did not. One explanation for this finding might be that in patients with higher tissue AGEs, collagen cross-links have been formed in the myocardium, which cannot be influenced by the current anti-hypertensive therapy. In contrast, in patients with lower tissue AGEs, the heart still has the ability to relax, and therefore skin-AF may be used to identify patients in whom an effect of blood pressure reduction on diastolic function can be expected.

This indicates the need for agents that can breakdown myocardial AGE cross-links to improve diastolic function. Such agents are AGE-cross-link breakers. Preliminary data from two small intervention trials with the AGE cross-link breaker alagebrium (ALT-711) have shown promising results in patients with chronic heart failure.^{19,20} In both trials ALT-711 led to an improvement in diastolic function, results that warrant further investigations using AGE lowering therapies in the treatment of diastolic dysfunction and/or heart failure. A prospective randomized, double-blind, placebo-controlled trial of the effects of alagebrium on exercise tolerance and diastolic function in 100 chronic heart failure patients is currently ongoing (BENEFICIAL trial, www.clinicaltrials.gov NCT00516646).²¹

In contrast with tissue AGE accumulation, plasma AGE levels were not associated with diastolic function in the present study. Both the LC-MS/MS method and the HPLC method used in our study to

assess plasma AGEs are currently considered as the most accurate methods available. In our opinion, our findings may suggest that plasma AGE levels do not adequately reflect tissue AGE accumulation. However, we cannot exclude the possibility that a power issue may explain the lack of correlation found between plasma AGEs and diastolic function. However, it would seem reasonable to assume that tissue AGEs are more closely related to diastolic function, because they are intrinsically linked with the actual pathological effects of AGEs (i.e. protein cross-linking).

Results of a recent study by van Heerebeek et al.²² challenged the idea that AGEs are of particular influence in patients with diastolic heart failure. They showed that diabetes especially increased AGE deposition in patients with a reduced ejection fraction. Shapiro et al.²³ challenged the idea that AGEs, through cross-linking impair vascular function. They showed that AGE accumulation was confined to the vasculature as were the effects of an AGE cross-link breaker. Although these data may provide some explanation of our negative findings on plasma AGEs, both studies only measured the accumulation of the non-cross-linking AGE Nε-(carboxymethyl)lysine (CML). Advanced glycation end-products are a heterogeneous group of compounds. Several different AGEs exist, some of which show cross-linking properties while others do not. Therefore, although the data are intriguing for definitive conclusions we will have to wait for the results of further investigations.²⁴

One limitation of our study is the fact that skin-AF was only measured in a sub-group of patients and therefore these results should be interpreted with caution. Also, although the autofluorescence measurements have been validated with tissue AGEs in the skin,^{14,15} the correlation between skin-AF and AGEs in the myocardium has so far not been studied. Therefore, the present results are only hypothesis generating, and should be confirmed in a larger prospective study. Another limitation is the fact that we included a population with only mild diastolic dysfunction. This could explain some of the negative findings of our study.

Conclusion

The ARB eprosartan did not decrease levels of AGEs in patients with hypertension and diastolic dysfunction. However, irrespective of the anti-hypertensive drugs used, patients with lower skin-AF at baseline showed a larger improvement in diastolic function in response to blood pressure reduction compared with those with higher skin-AF levels. Clinical trials using AGE lowering therapies are warranted to further explore the role of AGEs in the development and progression of diastolic dysfunction and subsequent heart failure.

Funding

This work was supported by an unrestricted research grant from Solvay Pharma (the Netherlands). J.W.L.H. is supported by a grant from the Netherlands Heart Foundation (2006T012). D.J.v.V. and A.A.V. are Clinical Established Investigators of the Netherlands Heart Foundation (D97-017 and 2006T37).

Conflict of interest: A.J.S. is associated with DiagnOptics BV, which manufactures autofluorescence readers. A.A.V. is associated with Synvita Therapeutics Inc. and Torrent Pharmaceuticals Ltd, both companies that develop AGE breakers. This study was not supported by either three companies and final approval was always by the first author (J.W.L.H.).

References

- Hartog JW, Voors AA, Bakker SJ, Smit AJ, van Veldhuisen DJ. Advanced glycation end-products (AGEs) and heart failure: Pathophysiology and clinical implications. *Eur J Heart Fail* 2007;**9**:1146–1155.
- Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part II: causal mechanisms and treatment. *Circulation* 2002;**105**:1503–1508.
- Zieman SJ, Kass DA. Advanced glycation endproduct crosslinking in the cardiovascular system: potential therapeutic target for cardiovascular disease. *Drugs* 2004;**64**:459–470.
- Hartog JW, Voors AA, Schalkwijk CG, Scheijen J, Smilde TD, Damman K, Bakker SJ, Smit AJ, van Veldhuisen DJ. Clinical and prognostic value of advanced glycation end-products in chronic heart failure. *Eur Heart J* 2007;**28**:2879–2885.
- Kass DA, Bronzwaer JG, Paulus WJ. What mechanisms underlie diastolic dysfunction in heart failure? *Circ Res* 2004;**94**:1533–1542.
- Smit AJ, Lutgers HL. The clinical relevance of advanced glycation endproducts (AGE) and recent developments in pharmaceuticals to reduce AGE accumulation. *Curr Med Chem* 2004;**11**:2767–2784.
- Hartog JW, Hummel YM, Voors AA, Schalkwijk CG, Miyata T, Huisman RM, Smit AJ, van Veldhuisen DJ. Skin-autofluorescence, a measure of tissue advanced glycation end-products (AGEs), is related to diastolic function in dialysis patients. *J Card Fail* 2008;**14**:596–602.
- Miyata T, van Ypersele dS. Angiotensin II receptor blockers and angiotensin converting enzyme inhibitors: implication of radical scavenging and transition metal chelation in inhibition of advanced glycation end product formation. *Arch Biochem Biophys* 2003;**419**:50–54.
- Saisho Y, Komiya N, Hirose H. Effect of valsartan, an angiotensin II receptor blocker, on markers of oxidation and glycation in Japanese type 2 diabetic subjects: Blood pressure-independent effect of valsartan. *Diabetes Res Clin Pract* 2006;**74**:201–203.
- Monacelli F, Poggi A, Storace D, Durante A, Traverso N, Viviani GL, Odetti P. Effects of valsartan therapy on protein glycoxidation. *Metabolism* 2006;**55**:1619–1624.
- Busch M, Franke S, Wolf G, Rohde RD, Stein G. Serum levels of the advanced glycation end products N-Carboxymethyllysine and pentosidine are not influenced by treatment with the angiotensin receptor ii type 1 blocker irbesartan in patients with type 2 diabetic nephropathy and hypertension. *Nephron Clin Pract* 2008;**108**:c291–c297.
- Persson F, Rossing P, Hovind P, Stehouwer CD, Schalkwijk C, Tarnow L, Parving HH. Irbesartan treatment reduces biomarkers of inflammatory activity in patients with type 2 diabetes and microalbuminuria: an IRMA 2 substudy. *Diabetes* 2006;**55**:3550–3555.
- Smilde TD, van Veldhuisen DJ, Navis G, Voors AA, Hillege HL. Drawbacks and prognostic value of formulas estimating renal function in patients with chronic heart failure and systolic dysfunction. *Circulation* 2006;**114**:1572–1580.
- Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, Alderson NL, Thorpe SR, Baynes JW, Gans RO, Smit AJ. Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 2004;**47**:1324–1330.
- Meerwaldt R, Hartog JW, Graaff R, Huisman RJ, Links TP, den Hollander NC, Thorpe SR, Baynes JW, Navis G, Gans RO, Smit AJ. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol* 2005;**16**:3687–3693.
- Teerlink T, Barto R, Ten Brink HJ, Schalkwijk CG. Measurement of Nepsilon-(carboxymethyl)lysine and Nepsilon-(carboxyethyl)lysine in human plasma protein by stable-isotope-dilution tandem mass spectrometry. *Clin Chem* 2004;**50**:1222–1228.
- Izuhara Y, Miyata T, Saito K, Ishikawa N, Kakuta T, Nangaku M, Yoshida H, Saito A, Kurokawa K, van Ypersele de SC. Ultrapure dialysate decreases plasma pentosidine, a marker of "carbonyl stress". *Am J Kidney Dis* 2004;**43**:1024–1029.
- Hartog JW, de Vries AP, Lutgers HL, Meerwaldt R, Huisman RM, van Son WJ, de Jong PE, Smit AJ. Accumulation of advanced glycation end products, measured as skin autofluorescence, in renal disease. *Ann NY Acad Sci* 2005;**1043**:299–307.
- Little WC, Zile MR, Kitzman DW, Hundley WG, O'Brien TX, deGroof RC. The effect of alagebrium chloride (ALT-711), a novel glucose cross-link breaker, in the treatment of elderly patients with diastolic heart failure. *J Card Fail* 2005;**11**:191–195.
- Thohan V, Koerner MM, Pratt CM, Torre GA. Improvements in diastolic function among patients with advanced systolic heart failure utilizing alagebrium (an oral advanced glycation end-product cross-link breaker). *Circulation* 2005;**112**:U620–U620. 2647 Suppl 2.
- Willemsen S, Hartog JW, Hummel YM, Posma JL, van Wijk LM, van Veldhuisen DJ, Voors AA. Effects of alagebrium, an advanced glycation end-product breaker, in patients with chronic heart failure: study design and baseline characteristics of the BENEFICIAL trial. *Eur J Heart Fail*; doi:10.1093/eurjhf/hfp207. Published online ahead of print 25 January 2010.
- van Heerebeek L, Hamdani N, Handoko ML, Falcao-Pires I, Musters RJ, Kupreishvili K, Ijsselmuiden AJ, Schalkwijk CG, Bronzwaer JG, Diamant M, Borbely A, van d V, Stienen GJ, Laarman GJ, Niessen HW, Paulus WJ. Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension. *Circulation* 2008;**117**:43–51.
- Shapiro BP, Owan TE, Mohammed SF, Meyer DM, Mills LD, Schalkwijk CG, Redfield MM. Advanced glycation end products accumulate in vascular smooth muscle and modify vascular but not ventricular properties in elderly hypertensive canines. *Circulation* 2008;**118**:1002–1010.
- Hartog JW, Willemsen S, Voors AA. Letter regarding article, "Advanced glycation end products accumulate in vascular smooth muscle and modify vascular but not ventricular properties in elderly hypertensive canines". *Circulation* 2009;**119**:e233.