Recent studies have identified that first-line renoprotective agents that interrupt the renin-angiotensin system not only reduce BP but also can attenuate advanced glycation end product (AGE) accumulation. This study used in vitro, preclinical, and human approaches to explore the potential effects of these agents on the modulation of the receptor for AGE (RAGE). Bovine aortic endothelial cells that were exposed to the angiotensin-converting enzyme inhibitor (ACEi) ramiprilat in the presence of high glucose demonstrated a significant increase in soluble RAGE (sRAGE) secreted into the medium. In streptozotocin-induced diabetic rats, ramipril treatment (ACEi) at 3 mg/L for 24 wk reduced the accumulation of skin collagen-linked carboxymethyllysine and pentosidine, as well as circulating and renal AGE. Renal gene upregulation of total RAGE (all three splice variants) was observed in ACEi-treated animals. There was a specific increase in the gene expression of the splice variant C-truncated RAGE (sRAGE). There were also increases in sRAGE protein identified within renal cells with ACEi treatment, which showed AGE-binding ability. This was associated with decreases in renal full-length RAGE protein from ACEi-treated rats. Decreases in plasma soluble RAGE that were significantly increased by ACEi treatment were also identified in diabetic rats. Similarly, there was a significant increase in plasma sRAGE in patients who had type 1 diabetes and were treated with the ACEi perindopril. Complexes between sRAGE and carboxymethyllysine were identified in human and rodent diabetic plasma. It is postulated that ACE inhibition reduces the accumulation of AGE in diabetes partly by increasing the production and secretion of sRAGE into plasma.
imental diabetic nephropathy and in samples from patients who had type 1 diabetes and participated in a clinical trial that explored early renoprotection with the ACEi perindopril (18).

Materials and Methods

Cell Culture Experiments

Bovine aortic endothelial cells (BAEC) (19) were cultured in modified Eagle’s medium (MEM), supplemented with nonessential amino acids, gentamicin, 10% FBS, and a low-glucose environment (1.1 mmol/L glucose) until confluence. BAEC then were randomized to either low or high glucose (30 mmol/L) until 0.5% FBS in the presence and absence of the active metabolite of the ACEi ramipril, ramiprilat (150 μmol/L; Aventis, Frankfurt, Germany); the AT1R antagonist valsartan (10 μmol/L); or the mitochondrial reactive oxygen species scavenger idebenone (10 μmol/L) for 7 d. On day 7, the cells then were lysed with ice-cold RIPA buffer (150 mmol/L NaCl, 1 mmol/L EDTA, 50 mM Tris-Cl [pH 7.5], 1% NP-40, and 0.25% deoxycholic acid) on ice. The RIPA buffer also contained the phosphatase inhibitors 1 mmol/L Na3VO4, 1 mmol/L NaF, and 30 mmol/L Na3P and a complete protease inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany). The protein concentrations of the lysates and cell culture supernatants (CCSN) were measured using the BCA protein assay (Pierce Biotechnology, Rockford, IL). Three independent experiments were carried out with essentially similar results.

Experimental Rodent Model

Diabetes was induced in male Sprague Dawley rats (200 to 250 g) by streptozotocin (intravenously, 50 mg/kg) in sodium citrate buffer (pH 4.5) after an overnight fast (20,21). Animals with plasma glucose concentrations >15 mmol/L 1 wk after induction of diabetes were included in the study. Sham-operated control animals (buffer only) were followed concurrently. Diabetic and control animals were randomized into groups (n = 10) that received either no treatment (D and C) or ramipril at a dose of 3 mg/L in drinking water (DACEi and CACEi) and followed for 24 wk. This dose was based on previous in vivo studies showing inhibition of the RAS as assessed by an increase in plasma renin activity as well as a reduction in AGE accumulation (10). Two units of Ultralente insulin (Ultratard HM; Novo Industries, Bagsvaerd, Denmark) were administered daily to dia-

age or 140/90 if younger than 40 yr (18). The exclusion criteria were nondiabetic renal disease, evidence of poor diabetic control (HbA1c >10%), cardiac failure, and systemic disease (18). All human procedures were in accordance with guidelines set by the Austin Hospital Human Ethics Committee and the National Health and Medical Research Council of Australia.

Plasma Low Molecular Weight AGE Analysis

Low molecular weight (LMW) AGE fluorescence was assayed in duplicate 20-μl plasma aliquots as described previously (22). Briefly, samples were deproteinized by addition of 0.15 M TCA and then delipidated using chloroform (23), followed by centrifugation and removal of the upper aqueous phase. Fluorescence of the supernatant was determined (Ex 370 nm, Em 440 nm) using an on-line HPLC injector (Waters, Milford, MA) and expressed per arbitrary protein unit at 280 nm.

Isolation of Skin Collagen and Analysis of AGE/ALE

Rat skin collagen was prepared as described previously (7). In brief, insoluble collagen was isolated from 1.5-cm² pieces of skin after removal of adventitious tissue with a razor blade and subsequent sequential extractions with 1.0 mol/L NaCl, 0.5 M acetic acid, and delipidation with chloroform:methanol (1:2). The collagen then was lyophilized and stored at −20°C until analysis of AGE/advanced lipoxidation end product (ALE) content.

The AGE/ALE Ne(carboxymethyl)lysine (CML) and Ne(carboxyethyl)-lysine were quantified by isotope dilution, selected ion monitoring gas chromatography–mass spectrometry (24), and normalized to their parent amino acid lysine. Pentosidine was analyzed by reverse phase–HPLC and was also normalized to lysine content (24).

Renal Cortical Fluorescence

Renal cortical tissue samples (100 mg wet weight) were acid hydro-
lyzed (6 M HCL for 24 h at 110°C) using the procedure of Stegemann and Stalder (25). After clarification with DEAE resin and neutralization, AGE fluorescence was determined as described previously (22) via a flow injection system adapted from Wrobel et al. (23) with a Waters 470 spectrophotometer (Ex 370 nm/Em 440 nm; Waters). AGE peptide fluorescence was expressed per protein density unit at 280 nm as described previously (22).

Immunohistochemistry for Renal CML

A modification of the ABC Ig enzyme bridge technique (26) was used for immunohistochemistry as described previously (22). The monoclonal AGE antibody 4G9, which was used in this protocol (1:500; Alteon Inc., Ramsey, NJ), recognizes CML (27). Negative control sections had the omission of the primary antibody. Positive control tissues were also included. Quantification of renal cortical immunostaining was completed by computer-aided densitometry (MCID-Video Pro-32; Bedford Park, SA, Australia), whereby a total of 20 fields (×100) were counted per section corresponding to a total kidney area of 6.08 mm². Ten animals per group were counted. Results were expressed as proportional area of positive staining (28).

Reverse Transcription–PCR

Three micrograms of total RNA extracted from each kidney cortex was used to synthesize cDNA with the Superscript first-strand synthesis system for reverse transcription–PCR (RT-PCR; Life Technologies BRL, Grand Island, NY). Gene expression for each of the sequences identified in Table 1 was analyzed by real-time quantitative RT-PCR
performed with the TaqMan system based on real-time detection of accumulated fluorescence (ABI Prism 7700; Perkin-Elmer, Foster City, CA) as described previously (29). The amplification was performed with the following time course: 2 min at 50°C and 10 min at 95°C and 40 cycles of 20 s at 94°C and 1 min at 60°C. Each sample was tested in triplicate; the average interassay coefficient of variation was 2.1%. Results were expressed relative to control kidneys, which were arbitrarily assigned a value of 1.

**Immunoprecipitation**

Samples of renal kidney cortex (100 mg wet weight) were microdissected and homogenized in 1 ml of cold neutral salt buffer (50 mmol/L Tris-Cl [pH 7.4], 150 mmol/L NaCl, and 5 mmol/L EDTA) that contained the protease inhibitors PMSF (1 mmol/L), leupeptin (10 μg/ml), and aprotinin (1 μg/ml) and then centrifuged at 3000 rpm for 1 h at 4°C. Protein concentrations in supernatants were determined by the BCA protein assay (Pierce Biotechnology). Tissue pools then were washed at least six times in wash buffer (50 mmol/L Tris-Cl [pH 7.4], 500 mmol/L NaCl, and 0.1% Tween 20). After the final wash, the AGE-binding capacity of protein that was immunoprecipitated (BLOTTO) followed by overnight incubations in primary antibody to total RAGE (goat anti-human RAGE; 1:1000, which recognizes all three splice variants). Bound antibodies were visualized by sequential incubation with biotinylated secondary antibody (1:15,000; DAKO, Carpinteria, CA), horseradish peroxidase–conjugated streptavidin (1:15,000; DAKO), and then autoradiography using an enhanced chemiluminescence kit (Pierce Biotechnology). Total band density from which loading control had been corrected was quantified using an imaging device with Optimas 6.2 Software (Optimas 6.2, Video Pro-32) associated with a JVC video camera and Olympus microscope. Loading controls for plasma samples was anti-albumin antibody (1:10,000; Sigma Chemical Co, St. Louis, MO). Results were expressed relative to control animals, which were arbitrarily assigned a value of 1.

**AGE/RAGE Complexes**

Pooled plasma samples (400 μl; n = 6) from untreated diabetic rats and patients with type 1 diabetes were concentrated ×10 with Amicon Microcon Filters (10000 MWCO; Millipore Corp.). Immunoprecipitation was performed as outlined above using either the N-terminal RAGE antibody (which recognizes the full-length and sRAGE splice variants; gift of Dr. E. Boel, Novo Nordisk) or total RAGE antibody (which recognizes all three splice variants; gift of Dr. Mike Neeper) diluted to 1 ml in binding buffer (50 mmol/L phosphate buffer [pH 7.2]) and rolled for 2 h at 4°C. Protein G slurry (50%) then was added, and the samples were rotated overnight. Samples then were centrifuged at 3000 rpm for 3 min, the supernatant was removed, and the gel slurry pellets were washed at least six times in wash buffer (50 mmol/L Tris-Cl [pH 7.4], 500 mmol/L NaCl, and 0.1% Tween 20). After the final wash, the gel slurry was resuspended in Laemmli S20 sample buffer (30), and β-mercaptoethanol was added to a final concentration of 0.1 mol/L. Suspensions were denatured at 95°C for 10 min before being subjected to SDS-PAGE (see below).

**Western Blotting**

For Western blotting, 5 μg of plasma protein or 50 μg of cell lysate protein was incubated at 65°C in S20 sample buffer that contained 60 mmol/L dithiothreitol for 10 min. CCSN were concentrated ×10 with Amicon Microcon Filters (10000 MWCO; Millipore Corp., Bedford, MA). These samples or 10 μl of immunoprecipitated sample prepared previously was separated on a 10% SDS–polyacrylamide electrophoresis gel (30). With the use of a semidy transfer tank (Bio-Rad Laboratories, Hercules, CA), proteins were transferred to a polyvinylidene difluoride membrane (Amersham Biosciences, Buckinghamshire, UK). Nonspecific binding sites were blocked for 1 h with 5% (wt/vol) nonfat milk powder in Tris-buffered saline (pH 7.4) and 0.05% Tween 20 (BLOTTO) followed by overnight incubations in primary antibody to total RAGE (goat anti-human RAGE; 1:1000, which recognizes all three splice variants). Bound antibodies were visualized by sequential incubation with biotinylated secondary antibody (1:15,000; DAKO, Carpinteria, CA), horseradish peroxidase–conjugated streptavidin (1:15,000; DAKO), and then autoradiography using an enhanced chemiluminescence kit (Pierce Biotechnology). Total band density from which loading control had been corrected was quantified using an imaging device with Optimas 6.2 Software (Optimas 6.2, Video Pro-32) associated with a JVC video camera and Olympus microscope. Loading controls for plasma samples was anti-albumin antibody (1:10,000; Sigma Chemical Co, St. Louis, MO). Results were expressed relative to control animals, which were arbitrarily assigned a value of 1.

<table>
<thead>
<tr>
<th>Probe Sequence</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Probe Start Position (bp)</th>
<th>GeneBank Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RAGE</td>
<td>TGTGCCATCTCTGC</td>
<td>TCCTGTTGGGACCGTAC</td>
<td>822</td>
<td>L33413</td>
</tr>
<tr>
<td>INT-RAGE</td>
<td>ATCCCAATTCAACCT</td>
<td>AGACGAGCTCCCCACACTAC</td>
<td>1341</td>
<td>L33413</td>
</tr>
<tr>
<td>ABS-RAGE</td>
<td>TGTCAGAACATCACGAC</td>
<td>CGGGTACTGGTTCTCTGCTGT</td>
<td>118</td>
<td>L33413</td>
</tr>
</tbody>
</table>

*Real-time reverse transcription–PCR probes and primers designed for the Taqman system by Primer Express for receptor for advanced glycation end products (RAGE) splice variants. INT-RAGE, probes and primers to the intracellular portion of RAGE; ABS-RAGE, probes and primers to the extracellular active binding site of RAGE; Total RAGE, probes and primers to the center of RAGE that recognize all three splice variants.

**Table 1. Real time RT-PCR probe and primer designs**

**Statistical Analyses**

Results are expressed as mean ± SD unless otherwise specified. Data for albuminuria were not normally distributed and therefore were analyzed following logarithmic transformation and are expressed as geometric mean ×/± tolerance factors. Analyses were performed by
ANOVA followed by post hoc analysis using Tukey least significant difference method, correcting for multiple comparisons. $P < 0.05$ was considered to be statistically significant.

**Results**

**Ramilprilat Increases Secretion of sRAGE in Cultured Endothelial Cells**

Western immunoblotting on BAEC lysates revealed a significant decrease in RAGE band density for groups that were treated with ramiprilat, with two bands resolved by SDS-PAGE at approximately 55 and 40 kD (Figure 1A). Only the band identified at 55 kD had AGE-binding affinity (FL-RAGE) as identified by Far Western immunoblotting, suggesting that the lower molecular weight band was N-RAGE (Figure 1B). In addition, there was a significant increase in C-truncated RAGE (sRAGE) at approximately 50 kD identified in the CCSN of BAEC that were treated with ramiprilat as compared with

![Figure 1](image-url)

*Figure 1.* Bovine aortic endothelial cells (BAEC) were exposed to low- and high-glucose environments in 0.5% FBS, in the presence and absence of ramiprilat. (A) Western immunoblotting of cell lysates with an antibody that recognizes all three splice variants of the receptor for advanced glycation end products (RAGE). (B) Far Western immunoblotting of cell lysates to assess the AGE-binding capacity of bands that were detected with RAGE antibody. (C) Western immunoblotting of cell culture supernatants (CCSN) from BAEC cultures with an antibody that recognizes all three splice variants of RAGE. (D) Quantification of soluble RAGE (sRAGE) in BAEC culture supernatants, representative of three separate experiments, arbitrary units per mg protein. (E) Far Western immunoblotting of CCSN from BAEC cultures to assess the AGE-binding capacity of bands that were detected with RAGE antibody. FL-RAGE, full-length RAGE; Ideb, idebenone; AT1a, valsartan. *$P < 0.001$ versus low glucose; †$P < 0.001$ versus high glucose; ‡$P < 0.01$ low-glucose angiotensin-converting enzyme inhibitor (ACEi) versus high-glucose ACEi; §$P < 0.005$ versus high-glucose ACEi.
untreated cells. This band also had AGE-binding capacity and was further increased upon exposure to high-glucose conditions (Figure 1C). This band was also identified in CCSN of cells that were treated with valsartan or idebenone under high-glucose conditions but only appeared significantly increased by idebenone as compared with the high-glucose control (Figure 1D). No other bands were identified on these membranes. These findings were confirmed in three separate experiments.

**Experimental Diabetic Nephropathy**

**Functional and Biochemical Parameters.** Diabetes was associated with increases in plasma glucose and HbA1c levels (Table 2). There was a significant decrease in body weight in diabetic animals, which was attenuated modestly by ACE inhibition. No changes in diabetes-induced increases in kidney to body weight ratios were seen with ACEi treatment. Untreated diabetic animals had increased systolic BP (SBP) and AER (Table 2). ACE inhibition reduced both SBP and AER to control levels. No increase in plasma cholesterol was seen at any time (Table 2).

**Tissue AGE Concentrations Are Increased with Experimental Diabetes and Reduced by ACEi.** Renal cortical CML levels were significantly increased (Figure 2A) in association with increased renal fluorescence (Figure 2B) in diabetic as compared with control animals. Treatment with ramipril prevented the diabetes-induced increases in renal CML and also attenuated the increase in renal fluorescence.

Both CML (Figure 2C) and pentosidine (Figure 2D) were increased in skin collagen of diabetic animals, whereas levels of Ne(carboxyethyl)lysine were unchanged (data not shown). ACE inhibition attenuated the increase in skin collagen CML without affecting skin collagen pentosidine levels (Figure 2, C and D).

**ACEi Can Modulate the Expression of Renal RAGE Splice Variants.** Real-time RT-PCR analysis of renal cortical gene expression demonstrated significant increases in the total expression of RAGE (Figure 3A) in both the untreated and the ACEi-treated rats.

Gene analysis for INT-RAGE (which corresponds to the C-terminus of RAGE, recognizing both full-length RAGE and N-truncated RAGE) showed increases with diabetes, which were prevented in the DACEi group (Figure 3B). No significant differences were detected between control groups.

Increases in ABS-RAGE (which recognizes both full-length RAGE and C-truncated/sRAGE at the N-terminus) were observed with diabetes in both untreated and ACEi-treated animals (Figure 3C). In addition, the control group that was treated with ACEi had significant increases in the expression of ABS-RAGE (Figure 3C).

**Renal RAGE Protein Isoform Expression Is Altered by ACEi.** Immunoprecipitation of renal cortical pools with an antibody that recognizes the C-terminal intracellular domain of RAGE yielded two major bands on SDS-PAGE (Figure 4A). The first band (approximately 58 kD) was representative of full-length RAGE as it had AGE-binding capacity as assessed by Far Western immunoblotting (Figure 4B). The second band at approximately 35 kD had no AGE-binding capacity and therefore was recognized as N-RAGE.

We used an antibody raised against the N-terminal extracellular domain of RAGE to immunoprecipitate full-length RAGE and C-truncated/sRAGE from renal cortical homogenates. This produced two major bands by SDS-PAGE, one at approximately 58 kD (Figure 4C) and a second, more intense band at approximately 40 kD. Each of these bands had AGE-binding capacity (Figure 4D).

**Increases in Circulating AGE Levels Are Accompanied by Decreases in Circulating sRAGE.**

**Experimental Diabetes.** Experimental diabetes induced a significant increase in plasma concentrations of LMW-AGE when compared with levels in control animals (Figure 5A). This increase in LMW-AGE was attenuated by ACE inhibition. Increases in LMW-AGE were accompanied by decreases in the levels of sRAGE (Figure 5B) in the plasma of diabetic animals.

**Patients with Type 1 Diabetes.** As has been described previously (18), perindopril provides superior protection from declining renal disease as compared with nifedipine for equivalent BP lowering. Plasma taken from patients with type 1 diabetes in the placebo group had significantly higher LMW-AGE concentrations at the completion of the study period than

**Table 2. Physiologic parameters for STZ rats at 24 wk**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 10)</th>
<th>Control + ACEi (n = 10)</th>
<th>Diabetes (n = 10)</th>
<th>Diabetes + ACEi (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>6.9 ± 0.3</td>
<td>6.9 ± 0.2</td>
<td>27.3 ± 2.5b</td>
<td>27.2 ± 1.0b</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>3.1 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>14.3 ± 0.8b</td>
<td>14.6 ± 0.6b</td>
</tr>
<tr>
<td>Mean SBP (mmHg)</td>
<td>127 ± 3</td>
<td>123 ± 1</td>
<td>147 ± 4b</td>
<td>123 ± 2c</td>
</tr>
<tr>
<td>AER (mg/24 h)</td>
<td>3.2 × 10⁻⁶</td>
<td>2.0 × 10⁻⁶</td>
<td>18.7 × 10⁻⁶b</td>
<td>2.2 × 10⁻⁶c</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>743 ± 19</td>
<td>700 ± 61</td>
<td>312 ± 21b</td>
<td>381 ± 70b,d</td>
</tr>
<tr>
<td>KW:BW ratio (×10⁻³)</td>
<td>5.8 ± 0.5</td>
<td>5.8 ± 0.4</td>
<td>13.5 ± 3.8b</td>
<td>12.9 ± 2.6b</td>
</tr>
<tr>
<td>Total plasma cholesterol (mmol/L)</td>
<td>2.1 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
</tbody>
</table>

aPhysiologic parameters for streptozotocin-induced diabetic rats at 24 wk. ACEi, angiotensin-converting enzyme inhibitors; SBP, systolic BP; AER, albumin excretion rate; KW, kidney weight; BW, body weight. Data are shown as mean ± SEM except for AER, which are shown as geometric mean × / ± tolerance factors.

bP < 0.001 versus control.

cP < 0.001 versus diabetic.

dP < 0.05 versus diabetic.
did patients who were treated with the ACEi perindopril (Figure 6A). By contrast, plasma sRAGE was increased in patients who received perindopril (Figure 6A). The patients who received placebo or nifedipine had no increase in plasma sRAGE; in fact, this was significantly decreased in these groups. There was an inverse correlation \((R^2 = 0.52, P = 0.047\); Figure 6B) between increasing plasma LMW-AGE and circulating levels of sRAGE in patients who were treated with perindopril.

**Complexes of RAGE- and CML-Modified Proteins Were Identified in Plasma**

Plasma from both diabetic rats and patients with type 1 diabetes demonstrated significant complexes between RAGE- and CML-modified proteins (Figure 6C). These complexes were detected with either CML or RAGE immunoprecipitation followed by detection with the alternate antibody. There were three bands evident at approximately 85, 50, and 33 kD.

**Discussion**

With the recent finding that there are in fact three splice variants of RAGE in humans (17), the evaluation of the role of this receptor in disease states has become even more complex. Our study has demonstrated the presence of each of the three splice variants in renal tissues from diabetic animals. The sum of total renal RAGE gene expression was found to be similar in diabetic and control rats. However, the relative distribution of the RAGE variants was complex, with increases in the full-length splice variant encoding the complete RAGE receptor (AGE-binding and downstream signaling ability) but with a decrease in tissue sRAGE gene expression. This pattern of RAGE receptor gene expression is likely to lead to an excess AGE “burden” whereby LMW-AGE were not being removed from the circulation by sRAGE and therefore are available for uptake by tissues, increasing end-organ AGE accumulation with subsequent activation of the full-length RAGE receptor. Indeed, diabetic animals in this study had significant increases in their AGE burden both in plasma and in tissues, in association with evidence of evolving renal disease. ACE inhibition altered the expression of renal RAGE splice variants with a reduction in the full-length splice variant mRNA in the context of increased expression of the splice variant encoding for sRAGE. This was associated with decreases in tissue CML and circulating LMW-AGE, thereby decreasing the overall AGE burden.

The nonhemodynamic effects of blockade of the RAS have increasingly been appreciated to play a role in mediating some of the renoprotection afforded by these agents. Indeed, our recent finding that ACE inhibition reduced the accumulation of AGE in experimental diabetic nephropathy has been confirmed in a number of further experimental studies (10,32). The mechanism by which this occurs remains unresolved. One mechanism has been excluded, because studies show that ACEi do not trap reactive carbonyl AGE precursors such as methylglyoxal (33,34), a common characteristic of the AGE formation inhibitors aminoguanidine, OPB-9195, and pyridoxamine (33,34). In part, effects on AGE reduction have been attributed to inhibition of the generation of free radicals that participate in the production of reactive carbonyls (9). In support of this, we have shown that ramipril reduced tissue nitrotyrosine levels in experimental diabetes (10), although
This is also seen with the AGE cross-link breaker ALT-711 (22). This is also supported by the in vitro experiments within our study in which treatment of endothelial cells with the mitochondrial reactive oxygen species scavenger idebenone showed some increase in sRAGE secreted into the CCSN. Therefore, part of the effect of ramipril on sRAGE may be via its antioxidant properties.

In this study, ramipril attenuated diabetes-induced increases in renal and tissue CML, a major AGE. CML has been identified as a ligand for the RAGE receptor (35). The relationship between AGE levels and RAGE expression has not been examined in detail. Typically, AGE and RAGE co-localize, and in states of increased AGE, there is also an increase in RAGE expression (36). Indeed, circulating complexes between sRAGE and RAGE were found within this study in both human and rodent plasma. Whether AGE per se promote RAGE expression is not known, although there has been some evidence supporting this in human vascular endothelial cells through NF-κB (37). Our group showed previously that NRK-52E cells that are exposed to CML-BSA overexpress RAGE, which in turn causes transition of these cells from their epithelial phenotype to myofibroblasts (20), a cell type that plays a major role in progressive renal scarring and fibrosis (38). This ability of AGE to modulate RAGE expression is likely to contribute to a pathologic state of chronic activation of the full-length RAGE receptor in disease states with elevated AGE levels, such as diabetes.

With evidence from the Diabetes Control and Complications Trial of patients with type 1 diabetes showing that skin CML levels are a major predictor of progression to diabetic complications (39), it is important to delineate the exact regulation not only of plasma but also of tissue components of the AGE pathway. Indeed, assessment of tissue collagen-associated AGE provides us with a more accurate record of historical levels of circulating AGE and glucose. The relative importance not only of sRAGE in regulating the level of circulating AGE but also of its postulated effect on tissue AGE levels remain to be determined. It therefore is tempting to speculate that the reduction seen in our study in the AGE, CML with ACE inhibition may ultimately influence the expression of RAGE.
A number of studies have suggested that administration of sRAGE is protective in a range of diabetic complications (13,15,16). Our study has identified a novel pathway for the production and secretion of sRAGE into the circulation. Untreated patients with type 1 diabetes had a significant decrease in circulating plasma sRAGE over the study duration in association with increases in circulating LMW-AGE when compared with patients who were treated with the ACEi perindopril. Although patients who were treated with nifedipine also had little increase in circulating AGE over the study period, it was interesting to note that these patients had a drop in circulating sRAGE and did not do as well as the patients who were on ACEi for equivalent BP lowering. These findings were also associated with progression of diabetic renal disease as assessed by an increase in AER over the study duration. By contrast, patients who were treated with perindopril had less renal disease with a significant reduction in albuminuria over the study period. Furthermore, plasma concentrations of LMW-AGE in these ACEi-treated patients had decreased over the 2-yr follow-up duration, whereas levels of “protective” sRAGE were significantly increased by perindopril therapy. We hypothesize that this increase in sRAGE could act as a mechanism to divert CML-modified peptides and other AGE from binding to the full-length RAGE receptor, acting as a “decoy” because this truncated form of the receptor has no downstream signaling capacity. One may speculate, at least in part, that the secretion of sRAGE into the circulation may originate from endothelial cells, a major site of RAGE expression (11), consistent with the significant increase in the concentration of sRAGE in the supernatants of bovine endothelial cells that were treated with the active metabolite of the ACEi ramiprilat. There was, however, significant sRAGE protein identified within renal cell homogenates particularly from ACEi-treated animals, which may also provide part of the pool of circulating sRAGE.
The AGE pentosidine can be generated from nonoxidative pathways that may not be blocked by ACE inhibition (40). In vitro studies have identified some effects of ACE inhibition on pentosidine (9), but these findings have not been confirmed in vivo. In addition, pentosidine is not a major ligand of RAGE; therefore, increasing levels of protective sRAGE in the plasma would more likely have no effect on reducing tissue pentosidine levels. It is unclear whether the lack of effect of ACEI in vivo on certain AGE such as pentosidine could partly explain why these agents do not totally prevent but only retard the rate of progression to ESRD in diabetes (41). There is also intracellular glycation occurring in diabetes, and this phenomenon is likely not to be readily affected by increases in circulating sRAGE levels. This is supported by the fact that although circulating LMW-AGE were normalized with ACE inhibition, tissue levels of CML in the diabetic animals were only partially attenuated by this treatment.

These findings lead us to conclude that ACEI play a pivotal role in reducing the burden of diabetic nephropathy as a result of advanced glycation and activation of RAGE. These results suggest that in the future, it is likely that therapies will confer superior renoprotection independent of their previously established mechanism of action, thereby further preventing progression to ESRD in diabetes.

Acknowledgments

This work was completed with support from the Juvenile Diabetes Research Foundation (JDRF), the National Health and Medical Research Council of Australia, and United States Public Health Service Grant DK-19971. J.F. is a JDRF Post-Doctoral Research Fellow. M.T. is a recipient of a Don & Lorraine Jacquot Fellowship. A.E.-O. is supported by a fellowship from the FRAXA Research Foundation.

We thank Gavin Langmaid for expert care of the animals throughout the study and Maryann Arnstein for technical expertise.

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See related editorial, “Angiotensin-Converting Enzyme Inhibition in Diabetic Nephropathy: It’s All the RAGE,” on pages 2251–2253.