Disparate effects on renal and oxidative parameters following RAGE deletion, AGE accumulation inhibition, or dietary AGE control in experimental diabetic nephropathy

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1Juvenile Diabetes Research Foundation Einstein Centre for Diabetic Complications, Baker International Diabetes Institute and Heart and Diabetes Research Institute, Melbourne; 2Department of Medicine, Austin and Northern Clinical Schools, University of Melbourne, Melbourne; 3Department of Immunology, Alfred Medical Research and Education Precinct, Monash University, Melbourne, Australia; and 4Department of Internal Medicine I and Clinical Chemistry, University of Heidelberg, Heidelberg, Germany

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Tan AL, Sourris KC, Harcourt BE, Thallas-Bonke V, Penfold S, Andrikopoulos S, Thomas MC, O’Brien RC, Bierhaus A, Cooper ME, Forbes JM, Coughlan MT. Disparate effects on renal and oxidative parameters following RAGE deletion, AGE accumulation inhibition, or dietary AGE control in experimental diabetic nephropathy. Am J Physiol Renal Physiol 298: F763–F770, 2010. First published December 16, 2009; doi:10.1152/ajprenal.00591.2009.—Advanced glycation end products (AGEs) and the receptor for AGEs (RAGE) generate ROS, and therefore this study evaluated the effects of RAGE deletion, decreasing AGE accumulation, or lowering dietary AGE content on oxidative parameters in diabetic nephropathy (DN). Control and diabetic male wild-type and RAGE-deficient (RAGE−/−) mice were fed high- or low-AGE diets, with two groups given the inhibitor of AGE accumulation, alagebrium chloride, and followed for 24 wk. Diabetic RAGE−/− mice were protected against albuminuria, hyperfiltration, glomerulosclerosis, decreased renal mitochondrial ATP production, and excess generation of both mitochondrial and cytosolic superoxide. Whereas glomerulosclerosis, tubulointerstitial expansion, and hyperfiltration were improved in diabetic mice treated with alagebrium, there was no effect on urinary albumin excretion. Both diabetic RAGE−/− and alagebrium-treated mice had an attenuation of renal RAGE expression and decreased renal and urinary AGE (carboxymethyllysine) levels. Low-AGE diets did not confer renoprotection, lower the AGE burden or renal RAGE expression, or improve cytosolic or mitochondrial superoxide generation. Renal uncoupling protein-2 gene expression and mitochondrial membrane potential were attenuated by all therapeutic interventions in diabetic mice. In the present study, diverse approaches to block the AGE-RAGE axis had disparate effects on DN, which has potential clinical implications for the way this axis should be targeted in humans.

advanced glycation; advanced glycation end products; receptor of advanced glycation end products

KEY CONTRIBUTORS TO THE PATHOGENESIS of diabetic nephropathy include advanced glycation (3) and oxidative stress (14, 28), which are thought to interact to exacerbate renal injury. In general, human studies with antioxidants have been disappointing (44), although end-organ protection has been demonstrated with antioxidants in models of experimental diabetes (21). Thus it is likely that conventional antioxidant therapy is not likely to have particular benefit as part of the strategy to reduce diabetic complications, including nephropathy. However, specific attenuation of the generation of reactive oxygen species (ROS), particularly within the mitochondria (28), and restoration of ATP generation (34) are postulated as desirable outcomes to prevent cell death and preserve renal function in diabetic nephropathy.

The excess accumulation of nonenzymatically modified proteins, namely, advanced glycation end products (AGEs), is implicated by a number of experimental studies including in vivo models of AGE infusion (6, 40) and strategies using a variety of chemically disparate inhibitors of AGE formation (9, 15, 36). Indeed, there is mounting evidence that almost all AGE inhibitors have beneficial common downstream effects on oxidative stress in diabetic nephropathy, including decreasing ROS production (37). Furthermore, glycation of mitochondrial proteins is thought to contribute to ROS generation in the diabetic kidney (35).

Reducing dietary AGE intake has also been reported to be therapeutically useful in diabetic renal disease (39, 45). This needs to be considered in the light of modern food processing, enriching the AGE content of foodstuff and the kidney being a major site for AGE excretion (24). The specific effects of reducing dietary AGEs on circulating oxidative parameters have been investigated in healthy adults (38) and in patients with type 2 diabetes (27). However, this link between AGEs and oxidative stress has not been directly investigated in diabetic nephropathy.

Interactions between AGEs and the receptor for AGEs, RAGE, are important for the development renal impairment in diabetes. Indeed, early experimental diabetic nephropathy is prevented with either “decoy” soluble RAGE, RAGE deletion (42), or by RAGE-neutralizing antibodies (12), whereas RAGE overexpression in diabetes worsens renal disease (43). These studies have primarily evaluated changes in renal function and structure, but the effects of in vivo RAGE modulation on mitochondrial metabolism and parameters of oxidative stress have not been extensively examined.

Hence, in the present study we have evaluated the contribution of the AGE-RAGE axis to renal mitochondrial oxidative stress and ATP generation using several different strategies:
first, RAGE deletion; second, inhibition of AGE accumulation; third, control of dietary AGE; and fourth, various combinations of these strategies in experimental diabetes.

METHODS

In vivo mouse models. Male wild-type (WT) C57BL/6J mice (17) and mice with a genetic deletion of RAGE on a C57BL/6J background (RAGE/-/-) (23) were randomized to receive a diet either high [HAGE; 101.9 nM lysine/100 mg carboxymethyllysine (CML), AIN-93G, baked at 160°C for 1 h; Specialty Feeds, Glen Forrest, Perth, Australia] or low in AGE content (LAGE; 20.9 nM lysine/100 mg CML, unbaked AIN-93G). WT and RAGE/-/- mice were injected with streptozotocin (55 mg/kg ip daily for 5 consecutive days, diabetic) (16) or sodium citrate buffer (control). After 10 days, plasma glucose concentrations were determined to ensure that the mice included were indeed overtly diabetic (>15 mM; seen in >98% of mice). Two groups of WT diabetic mice (on a high- or low-AGE diet) were randomized to receive the inhibitor of AGE accumulation, alageburim chloride [ALT-711; 4,5-dimethyl-3-(2-oxo-2-phenylethyl) thiazolium chloride; Synvista Therapeutics, Ramsey, NJ] by oral gavage at 1 mg·kg⁻¹·day⁻¹. The diets and water were provided ad libitum, and mice were housed in specific pathogen-free housing conditions with exposure to 12:12-h light-dark cycles. Mice were followed for 24 wk (n = 10 mice/group). All animal studies were performed in accordance with guidelines approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee and the National Health and Medical Research Council of Australia. At the end of the study, glycated hemoglobin (GHB) was determined by high-performance liquid chromatography (HPLC) (4). Systolic blood pressure was estimated by computerized, noninvasive tail-cuff plethysmography in conscious mice (22). Urinary albumin excretion rate (AER) was determined by ELISA (Bethyl Laboratories, Montgomery, TX) (5), and creatinine in urine and plasma was determined by HPLC (Agilent HP1100 system; Hewlett Packard, Böblingen, Germany) according to the Animal Models of Diabetic Complications Consortium guidelines (11). Glomerular sclerotic index (GSI) and tubulointerstitial area (TIA) were assessed in paraffin-embedded periodic acid-Schiff (PAS)-stained sections by using a semiquantitative method and point-counting technique, respectively, as previously described (15).

Real-time RT-PCR. Less than 2 μg of total RNA were extracted from the left kidney cortex and used to synthesize cDNA (Superscript First-Strand Synthesis kit for RT-PCR; Invitrogen Life Technologies, Carlsbad, CA). Gene expression of RAGE and UCP2 were 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, Waltham, MA) as described previously (13) and using the following sequences: RAGE forward primer, 298GCTGTAAGCTGGTGTTCAAGACAGA298, reverse primer, 298CCCTTAACAGTCTAGCCAA298, and probe, 6-FAM 298CCACAGCCGGATG298, and UCP2 forward primer, 298CCGTAAGTGCGGTCTAAGG298, reverse primer, 298GCTTCTCGAGAGTGATACCTTGAG298, and probe, 6-FAM 298AATGGAACACAGCCTCA298. Results are expressed relative to control WT kidneys, which were assigned an arbitrary value of 1.

Renal fractionation. The right kidney cortex was homogenized in extraction buffer (20 mM HEPES, 1 mM EGTA, 210 mM sucrose, and 70 mM sucrose, pH 7.2). Homogenates were centrifuged at 1,000 g for 10 min, and the resulting supernatant was centrifuged at 10,000 g for 20 min. The pellet was resuspended in extraction buffer as the mitochondrial fraction, whereas the supernatant was centrifuged at 100,000 g for 1 h. The resulting supernatant contained the cytosolic fraction, whereas the pellet was resuspended in extraction buffer and further centrifuged at 100,000 g for 1 h. The resulting supernatant contained the membranous fraction. Total protein content of the renal fractions was determined using the bicinechonic acid method (Pierce, Rockford, IL).

RAGE and ligands. ELISAs were used to determine concentrations of RAGE in membranous fractions (R&D Systems, Minneapolis, MN) and high-mobility group box 1 (HMGB1) in plasma and membranous fractions (Shino-Test, Shagamihara, Japan) according to the manufacturer’s instructions. CML concentrations in urine, plasma, and membranous fractions were determined as previously described (5). CML clearance was calculated using the following formula: CML(serum)/(urine volume)/CML(serum).

Glycolytic and oxidative parameters. Lactate and pyruvate concentrations in cytosolic fractions were determined by ELISA according to the manufacturer’s instructions (Biovision, Mountain View, CA). Lucigenin-enhanced cytosolic and mitochondrial superoxide production in renal cortices (5) and ATP production in mitochondrial fractions (Molecular Probes, Alameda, CA) (10) were determined as previously described. Manganese superoxide dismutase (MnSOD) activity was measured in mitochondrial fractions using a commercially available assay (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions. NADH content was measured in cytosolic and mitochondrial fractions by depleting of the oxidized NAD⁺ isofrom, followed by a modification of the method originally described by Nisselbaum and Green (29) and described by Coughlan et al. (5). Membrane potential in mitochondrial fractions was measured using the MitoProbe JC-1 (6-tetraiodo-1,1,3,3-tetraethylbenzimidazocarbocyanine iodide) assay kit for flow cytometry (Molecular Probes). Mitochondria (80 μg/ml) were incubated for 20 min in extraction buffer at 37°C with 0.8 μM JC-1. A negative (no JC-1) and positive control (50 μM CCCP) were also added. Suspensions were washed, pelleted, and resuspended in PBS before analysis by flow cytometry (minimum 10,000 events/sample; FACSCalibur; BD Biosciences, San Jose, CA). The ratio of a decline in red to increased green fluorescence intensity was determined.

Statistical analysis. All statistical analyses were performed using GraphPad Prism 5.00 for Windows (La Jolla, CA) and one-way ANOVA with Tukey’s post-test analysis and two-tailed unpaired t-tests where appropriate. Unless otherwise specified, results are means ± SE and P < 0.05 was considered statistically significant.

RESULTS

Biochemical and metabolic parameters. Diabetes-induced increases in glycated hemoglobin were attenuated with alageburim therapy, particularly in conjunction with a low-AGE diet (Table 1). Total kidney-to-body weight ratio was increased with diabetes in all mice (Table 1). Diabetes induced a decrease in body weight that was modestly improved in low-AGE-fed RAGE/-/- mice (Table 1). Caloric intake was increased with diabetes and moderately improved by a low-AGE diet (Table 1). Diabetes induced an increase in water intake that was further elevated in low-AGE-fed mice with alageburim treatment or RAGE deficiency (Table 1). There was a significant decline in mean systolic blood pressure (SBP) with diabetes that was only normalized with alageburim therapy (Table 1).

Renal functional and structural parameters. At both weeks 12 and 24 of the study, diabetes was associated with an increased creatinine clearance, which was ameliorated by all interventions (Fig. 1, A and B). Urinary albumin excretion rate (AER) was elevated in diabetic mice compared with controls, although this increase in AER was not seen in diabetic RAGE-deficient mice (Fig. 1C). Diabetes induced elevations in GSI that were normalized in RAGE-deficient mice and with alageburim therapy but not by a low-AGE diet alone (Fig. 1D). TIA was also increased with diabetes, and this was attenuated by...
alagebrium therapy alone or modestly by combination therapy with RAGE deficiency and a low-AGE diet (P = 0.052 for DAlt-LAGE vs. D; Fig. 1E).

RAGE and ligand concentrations. Expression of RAGE in renal cortical membranes (Fig. 2A) and renal cortical RAGE gene expression (Table 1) were increased by diabetes but were unaffected by any treatment except for alagebrium monotherapy. Both RAGE protein and gene expression were undetectable in RAGE-deficient mice (Fig. 2A and Table 1, respectively). Plasma CML concentrations were decreased in all diabetic mice, and this was attenuated in RAGE-deficient mice (Fig. 2B). Increases in urinary CML concentrations seen with diabetes were ameliorated in RAGE-deficient mice by alagebrium therapy alone and when combined with a low-AGE diet (Fig. 2C). The clearance of CML modified proteins was also calculated as per creatinine clearance. Diabetic control mice had a significant increase in the clearance of CML-modified proteins, which was improved with each of the therapeutic interventions, suggesting a reduced “body” CML load in the treatment groups (Fig. 2D). Renal membranous CML content

Table 1. Biochemical and metabolic parameters at week 24.

<table>
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<th>D</th>
<th>DAlt</th>
<th>D-RAGE ''+''</th>
<th>C</th>
<th>D</th>
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Values are means ± SE for blood pressure, receptor of advanced glycation end product (RAGE) expression, and high mobility group box 1 (HMGB1) content and means ± SD for glycated hemoglobin, kidney weight/body weight, body weight, and calorie and water intake; n = 8–10 per group for wild-type (WT) mice, and n = 18–20 for RAGE-deficient (RAGE''−/−'') mice. C, control mice; D, diabetic mice; DAlt, WT diabetic mice + alagebrium; D-RAGE''−/−'', diabetic RAGE-deficient mice; nd, not detected. *P < 0.001; †P < 0.01; ‡P < 0.05, C vs. D mice on high-advanced glycation end product (AGE) diet. †P < 0.001; *P < 0.01; ‡P < 0.05 vs. D. DAlt or D-RAGE''−/−'' mice on high-AGE diet vs. respective group on low-AGE diet.

Fig. 1. Renal functional parameters. Unless otherwise specified, high-advanced glycation end product (AGE) diets were fed to control mice (C), diabetic mice (D), diabetic mice treated with the AGE-lowering therapy alagebrium (DAlt), diabetic receptor for AGE (RAGE)-deficient (RAGE''−/−'') mice (D+R), or diabetic mice fed a low-AGE diet (D+LAGE). Creatinine clearance (CrCl) was determined by HPLC at week 12 (A) and week 24 (B). Urinary albumin excretion rate (AER; C), glomerulosclerotic index (GSI; D), and tubulointerstitial area (TIA; E) were determined at week 24. Values are means ± SE; n = 8–10 per group. *P < 0.05 vs. C. †P < 0.05; ‡P < 0.01 vs. D. #P < 0.05 vs. DAlt.
was also increased with diabetes (Fig. 2E) and ameliorated with alagebrium therapy or in RAGE−/− mice. Diabetes also decreased the concentrations of the RAGE ligand HMGB1 in plasma compared with control mice, but this remained unaffected by any treatments (Table 1). Within the renal cortex, membrane expression of HMGB1 remained unchanged among control and diabetic mouse groups (Table 1).

Cytosolic glycolytic parameters. Lactate-to-pyruvate ratios measured in renal cortical cytosol were increased by diabetes but were not altered with any of the therapeutic interventions (Fig. 3A). Renal cortex from diabetic mice also had elevated cytosolic NADH concentrations measured in renal cortices taken from diabetic mice; this was attenuated with RAGE deletion alone but not by alagebrium or a low-AGE diet (Fig. 3B). Excessive cytosolic superoxide was measured in renal cortices taken from diabetic mice; this was attenuated with RAGE deletion alone but not by alagebrium or a low-AGE diet (Fig. 3C).

Mitochondrial function. Mitochondrial NADH content in the kidney was modestly decreased by diabetes and improved by alagebrium and a low-AGE diet (Fig. 4A). Mitochondrial membrane potential in renal cortical mitochondria was disrupted with diabetes and was ameliorated with all treatments (Fig. 4B). Renal cortical mitochondria from diabetic mice also had a decreased capacity for ATP production, and this was only attenuated in RAGE−/− mice (Fig. 4C). Furthermore, diabetes also induced excess production of superoxide in renal mitochondria that was normalized with RAGE deletion, alagebrium, or a low-AGE diet (Fig. 4D). Renal mitochondrial MnSOD activity significantly declined with diabetes; this was only restored by alagebrium therapy and was unaffected by any treatment (Fig. 4E). UCP2 gene expression in diabetic renal cortices was also elevated compared with control mice, and this was abrogated with all therapies except for a low-AGE diet alone (Fig. 4F).

**Discussion**

This group of studies suggests that there are key differences in targeting various components of the AGE-RAGE pathway. The effects on cellular energy production and superoxide generation with each of the therapeutic interventions studied directly related to their renoprotective effects. Within the present study, the most important factor associated with AER was the maintenance of renal mitochondrial ATP production, most evident in RAGE−/− mice, which had preservation of ATP and amelioration of albuminuria. Not surprisingly, RAGE expression and/or signaling is known to be important for diabetes-induced renal damage (26). It therefore seems intuitive that the greatest improvements in renal parameters were seen with alagebrium and in RAGE-deficient mice, each of which had lower expression of renal membranous RAGE. Also, given that these two groups showed much lower cytosolic concentrations (or generation) of CML-modified proteins, it is likely that the tissue concentrations of AGEs, in particular CML, exert greater modulatory effects on membranous RAGE expression than circulating CML concentrations. In addition,
Alagebrium and low-AGE diets increased blood pressure in diabetic mice, which also may have countered some of their positive effects on other parameters, whereas, interestingly, diabetic RAGE−/− had the lowest systolic blood pressure. Indeed, we previously showed that alagebrium normalizes lowered blood pressure in streptozotocin diabetic rats (15) and apoE−/− mice (16). It is well accepted that rodents with streptozotocin-induced diabetes have lower systolic blood pressure. Interestingly, the reasons for this have not been fully defined; however, it is thought that this may be one of the reasons for the relatively slow progression of renal disease in these models. Whether alagebrium has added capacity to facilitate the transport of CML-modified proteins into the urine given its much higher urinary concentration is worth investigation in the future but was beyond the scope of these studies, but this may also partly explain its superior renoprotective effects over low-AGE diets in the present study. Indeed, the clearance of CML into the urine has been previously shown in mouse models of type 2 diabetes (31).

Early diabetes-induced hyperfiltration, as assessed by creatinine clearance, was improved by all treatments, namely, RAGE deficiency, alagebrium therapy, and a low-AGE diet, although combinations of these different strategies did not confer superior protection. Not surprisingly, in humans there is a close relationship between renal function, including proteinuria and glomerular filtration rate and cardiovascular events (2, 8, 16). In support of this, beneficial renal outcomes with RAGE deficiency (26, 42), a low-AGE diet (45), and alagebrium therapy (15) have all been previously described in models of experimental diabetes; however, chronic renal functional and structural changes with diabetes have not been previously assessed in RAGE−/− mice or in mice fed a low-AGE diet. It is surprising that although beneficial renoprotective effects were seen in both diabetic RAGE−/− and alagebrium-treated mice, this was not the case for those diabetic mice fed a low-AGE diet. Indeed, there were no significant improvements in AER, glomerulosclerosis, or tubulointerstitial accumulation identified with AGE dietary restriction as has been described previously. This is most likely due to the short duration of diabetes used in previous studies (45). In addition, our own previous studies have shown beneficial effects of alagebrium on AER in streptozotocin diabetic rats (15), which were not seen in alagebrium–treated diabetic mice in the present study. This may have been due to the differences in dosages used in these studies, i.e., 10 mg·kg−1·day−1 previously vs. 1 mg·kg−1·day−1 in the present study. Furthermore, there is some evidence that urinary albumin excretion has limitations in prediction of progressive renal disease in diabetes (33, 46). There are also some differences in streptozotocin diabetes between rats and mice. Rats alone become insulin dependent, and therefore some effects of AGE-lowering therapies on residual insulin production in mouse models cannot be ruled out. Indeed, previously, diets low in AGE content were shown to improve pancreatic function in mouse models of both type 1 (32) and type 2 diabetes (18), although these effects were difficult to assess in our streptozotocin mouse model, since most pancreatic islets have been destroyed in this model and we did not see effects of the low-AGE diet on GHb concentrations. It is also likely that the maintenance of cytosolic NADH content seen in low-AGE-fed diabetic mice, as the result of sustained glycolysis (Fig. 5), could be detrimental to cellular and mitochondrial function by continually supplying excess fuel to the mitochondria and facilitating excess cytosolic superoxide and AGE generation, which were evident in diabetic mice.

Maintenance of cellular glycolysis, however, is also critical for ATP production. Earlier studies have shown that diabetes increases the cytosolic lactate-pyruvate ratio in the kidney (19, 30), which is consistent with our study. Nevertheless, since the cytosolic lactate-pyruvate ratios were not improved by any of
the treatments used, specific modulation of glycolysis may not be directly involved in the benefits afforded by the therapies tested. We cannot rule out the possibility, however, that the availability of other glycolytic intermediates and products are not relevant to preservation of kidney function in diabetes. Specifically, the diabetes-induced elevations in cytosolic NADH concentrations were normalized by alagebrium therapy and RAGE deficiency, which showed the greatest renoprotective benefits. This is also consistent with previous findings (20). The significance of this, however, has to be further investigated, because cytosolic NADH concentrations are influenced by many factors, including the polyol pathway, anaerobic fermentation, protein kinase C, and mitochondrial NADH uptake (1). Indeed, our previous studies have shown that maintenance of the supply of NADH to mitochondria is critical for sustained mitochondrial superoxide generation in the diabetic kidney (5). In the present study, we showed that renal mitochondrial content of NADH was modestly decreased with diabetes, suggesting its use by the respiratory chain, although this is likely in this environment to be contributing to the mitochondrial superoxide generation.

Excess generation of mitochondrial superoxide is widely postulated to be the most important contributor to the pathogenesis of diabetic complications (28). The diabetes-induced increase in cytosolic superoxide was normalized by RAGE deletion and alagebrium therapy to a lesser degree. Indeed, a previous study has identified a central role for NADPH oxidase in AGE-mediated ROS generation (41). Not surprisingly, in the present study, mitochondrial superoxide generation was also increased with diabetes as consistent with previous studies (5, 6). Interestingly, RAGE−/− mice had the most benefits on AER and preservation of ATP generation, in addition to amelioration of excess mitochondrial superoxide production. Our data are in agreement with previous studies, where diabetes modulated mitochondrial respiration (25, 34) and increased

Fig. 4. Renal mitochondrial function at week 24. Mice were fed high-AGE diets unless otherwise specified. Renal cortical mitochondrial NADH content (A), membrane potential determined by JC-1 and flow cytometry (B), ATP production (C), NADH-dependent superoxide generation (D), and MnSOD (SOD2) activity (E) were determined. F: UCP2 gene expression in renal cortex as determined by real-time RT-PCR. Values are means ± SE; n = 8–10 per group. *P < 0.05 vs. C. †P < 0.05; ‡P < 0.01 vs. D.

Fig. 5. Schematic diagram of the interactive oxidative pathways examined. O2−, superoxide radical; H2O2, hydrogen peroxide radical; Ψm, mitochondrial membrane potential; e−, electrons in the electron transport chain.
mitochondrial membrane potential (Fig. 5). This group of events could indeed represent a compensatory mechanism that is attempting to maintain cellular ATP production. However, increased expression of uncoupling proteins (7), as was found with diabetes in the present study, may favor electron-flux dissipation as heat and superoxide (Fig. 5), rather than sequential passage of electrons to ATP synthase. Alagebrum therapy also decreased mitochondrial superoxide production, consistent with our previous studies in rats (5, 6), in the context of significant improvements in MnSOD activity. In addition, those diabetic mice treated with alagebrum had improved creatinine clearance and renal structural parameters, suggesting significant renoprotection by lowering oxidative damage within mitochondria.

Overall, in the present study, RAGE deficiency alone was uniquely renoprotective in normalizing albuminuria. We suggest that one key benefit of RAGE deficiency was its ability to restore the diabetes-induced decrease in renal ATP production (Fig. 5). This is further supported by the lack of protective effects on ATP generation and albuminuria with either alagebrum or a low-AGE diet. Interestingly, however, alagebrum therapy normalized creatinine clearance and improved renal structural parameters, which are also important determinants of progressive renal disease in diabetes and of macrovascular disease. Together, these data suggest that modulation of RAGE or its potential ligands, AGEs, have differential effects on renal function in experimental nephropathy that warrants further investigation, since target within this axis are under active investigation for clinical translation to human diabetic nephropathy.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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