Advanced glycation endproducts in the pathogenesis of chronic kidney disease

Naila Rabbani and Paul J. Thornalley
Warwick Medical School, Clinical Sciences Research Laboratories, University of Warwick, University Hospital, Coventry CV2 2DX, U.K.

Correspondence to: Professor Paul J Thornalley, Clinical Sciences Research Laboratories, Warwick Medical School, University of Warwick, University Hospital, Clifford Bridge Road, Coventry CV2 2DX, U.K. Email: P.J.Thornalley@warwick.ac.uk Tel +44 7696 8594 Fax +44 24 7696 8653

Word count: abstract 249; main text 3952.

Abstract

Advanced glycation endproducts (AGEs) are stable post-translational modifications of proteins formed by the spontaneous reaction with glucose and related metabolites. Important AGEs quantitatively are methylglyoxal (MG)-derived hydroimidazolone MG-H1, N_{ε}-carboxymethyl-lysine (CML) and glucosepane. They contribute to the development of chronic kidney disease (CKD). Cellular proteolysis of AGE-modified proteins forms AGE free adducts, glycated amino acids, which are cleared by the kidneys and excreted in urine. Dietary AGEs mainly supplement the endogenous flux of AGE free adduct formation. AGE free adducts accumulate markedly in plasma with decline in glomerular filtration rate. A key precursor of AGEs is the dicarbonyl metabolite, MG, which is metabolised by glyoxalase 1 (Glo1) of the cytoplasmic glyoxalase system. Proteins susceptible to MG modification are called collectively the "dicarbonyl proteome". Abnormal increase of MG "dicarbonyl stress" and is a characteristic of CKD, driven by down regulation of renal Glo1, increasing flux of MG-H1 formation. Protein inactivation and dysfunction linked to the dicarbonyl proteome contributes to CKD development. The receptor for AGEs, RAGE, is important in development of CKD but its interaction with AGEs *in vivo* remains enigmatic; other ligands and ternary complexation may be influential. Prevention of diabetic kidney disease (DKD) by overexpression of Glo1 in transgenic animal models has stimulated the development of small molecule inducers of Glo1 expression, "Glo1 inducers", to prevent AGE formation. *trans*-Resveratrol-hesperetin combination therapy is a Glo1 inducer. In clinical trial it gave a profound improvement in insulin resistance and vascular inflammation. It may find future therapeutic application for treatment of DKD.

Advanced glycation endproducts

Advanced glycation endproducts (AGEs) are a group of compounds formed by the non-enzymatic reaction of reducing sugars and related metabolites with proteins and amino acids. The process is called glycation or the Maillard reaction. Major precursors of AGEs *in vivo* are the early-stage glycation adduct, N_{ε}-fructosyl-lysine (FL), and dicarbonyl metabolites methylglyoxal (MG), glyoxal and 3-deoxyglucosone (3-DG).[1] Major AGEs quantitatively are MG-derived hydroimidazolone MG-H1 and FL-derived N_{ε}-carboxymethyl-lysine (CML) and the crosslink glucosepane - Figure 1. AGEs are formed as glycated amino acid residues of proteins which are conventionally called "AGE residues" of proteins, although they are often called "protein-bound AGEs" in renal research. AGE-modified proteins are degraded to related glycated amino acids, called AGE free adducts. AGEs are also formed from glucose osmolyte, MG and other glucose degradation product dicarbonyls absorbed from thermally processed dialysis fluids in renal replacement therapy. AGEs may also be absorbed from glycated proteins in food, mainly as AGE free adducts. AGEs may be quantified robustly by stable isotopic dilution analysis liquid chromatography-tandem mass

spectrometry (LC-MS/MS).[1] AGEs represent relatively long-lived and potentially damaging post-translational modification of proteins. They are mostly damaging through modification of functional domains of proteins, producing protein inactivation or dysfunction.

Herein we review evidence of protein-derived AGEs. There are also AGEs formed by MG and glyoxal modification of nucleotides and basic phospholipids, phosphatidylethanolamine and phosphatidylserine.[2, 3] These have been little-studied in chronic kidney disease (CKD)[4] and so the coverage below focusses on protein-derived AGEs.

The formation of AGEs is suppressed by enzymatic metabolism of the precursor glycating agents or glycation adduct, FL. MG and glyoxal are metabolised mainly by the cytoplasmic glyoxalase system. The glyoxalase system consists of 2 enzymes, glyoxalase 1 (Glo1) and glyoxalase 2 and a catalytic amount of reduced glutathione (GSH). Glo1 catalyses the GSH-dependent metabolism of MG to S-D-lactoylglutathione, and glyoxalase 2 catalyses the hydrolysis of S-D-lactoylglutathione to D-lactate, reforming GSH consumed in the Glo1 catalysed step[5] – Figure 2. 3-DG and likely also 3,4-dideoxyglucosone-3-ene (3,4-DGE) found in thermally processed peritoneal dialysis (PD) fluids with glucose osmolyte are metabolised by aldoketo reductases (AKRs) and aldehyde dehydrogenases. The kidney has a very high activity of aldose reductase, *ca.* 2% total protein, in the inner medulla which primarily reduces glucose to sorbitol to counter high extracellular osmotic pressure.[6] Steady-state levels of FL residues are suppressed by enzymatic metabolism by fructosamine-3-phosphokinase, resulting in de-glycation of precursor lysine residues.[1]

Dicarbonyl stress

Dicarbonyl stress is the abnormal accumulation of MG and related dicarbonyl compounds, leading to increased AGE formation and related cell and tissue dysfunction. Dicarbonyl stress is a driver of CKD development – as evidenced by development of nephropathy in Glo1 deficient mice.[7] Dicarbonyl stress may also be consequence of renal failure, as evidenced by dicarbonyl stress with loss of clearance in bilateral nephrectomised rats.[8] Dicarbonyl stress occurs in patients with CKD,[5] including accumulation of MG without increase in D-lactate in non-diabetic subjects.[9] D-Lactate is a marker of flux of formation of MG.[10] This suggests the driver of dicarbonyl stress in CKD is down regulation of Glo1 rather than increased formation of MG in non-diabetic subjects. Decreased urinary excretion of MG is not a major contributing factor since little MG is excreted – although this may be more important for 3-DG.[10] Down regulation of Glo1 in the kidney is a common feature of experimental diabetic nephropathy and diabetic kidney disease (DKD). It may be driven by decreased Glo1 expression in response to hypoxia-inducible factor-1α and inflammatory signalling conflicting with transcription factor Nrf2, and by increased proteolysis. Overexpression of Glo1 prevented renal senescence[11] and development of diabetic nephropathy[7, 12] – the latter even when only in endothelial and tubular epithelial cells.[7] This suggests reversing renal down regulation of Glo1 may provide a new route to therapy.[10]

The flux of MG formation in a healthy adult human subject is *ca.* 3 mmol MG per 24 h and >99% is normally metabolised enzymatically. MG concentration of PD fluids, 2 – 7 μM, is therefore not a major increment to MG exposure. For 3-DG, the flux of formation is *ca.* 0.13 mmol 3-DG per 24 h and *ca.* 90% is normally metabolised enzymatically with *ca.* 10% excreted. PD fluids containing 100 – 400 μM 3-DG increase exposure to 3-DG markedly, although 3-DG has *ca.* 200-fold lower reactivity that MG.[5, 10]

Accumulation of AGEs in renal failure – the profound increase of AGE free adducts

AGE free adducts are the major form by which AGEs are eliminated from the body. Decreased clearance in CKD markedly influences the plasma concentrations of AGE free adducts.[8, 13] The increase of AGE free adducts in clinical renal failure, studied in patients

receiving hemodialysis (HD) and PD renal replacement therapy was 4 - 40 fold whereas the increase in AGE residues of plasma protein was 2 – 5 fold.[13] Drivers of AGE free adduct accumulation are increased flux of formation of AGEs and decreased clearance. The flux of formation of AGEs is indicated by the total excretion of AGE free adducts in dialysate and urine. In PD patients, flux of AGE formation was increased markedly with respect to healthy controls: 9-fold for MG-H1 and 2-fold for CML, pentosidine and 3-DG-derived hydroimidazolones 3DG-H. The flux of excretion of MG-H1 free adduct in PD patients was 4 – 713 fold higher than of other AGEs, indicating MG-H1 is a dominant AGE in renal failure.[13]

AGE residue contents of plasma protein have been studied as biomarkers of mortality risk in renal failure with contrary outcomes or low marginal increased relative risk.[14, 15] Plasma protein AGEs, such as MG-H1 and 3DG-H, may be increased by dicarbonyl stress;[13] CML and glucosepane may be increased by elevated FL residue precursor and/or decreased FL metabolism;[16-18] and pentosidine residue content may be increased by elevated pentosephosphate pathway activity providing increased level of the pentose precursor.[19] AGE residue content of plasma protein is also influenced by decreased residence time of albumin in the vascular compartment by albuminuria,[20] increased transcapillary escape rate – influenced by hypertension and atherosclerosis;[21] and also by decreased albumin synthesis and catabolism.[22] These confounders suggest AGE residue content of plasma protein has a complex relationship with clinical outcomes in CKD.

An indication of increased AGE formation in the kidney may be gained by measuring total body flux of formation of AGEs, or surrogate measures thereof: such as urinary or dialysate flux of AGE free adducts, plasma AGE free adduct concentration corrected for decline in glomerular filtration rate (GFR) in CKD stages G1 – G4, and plasma AGE free adduct concentration immediately prior to a dialysis session in HD patients or dialysis fluid exchange in PD patients. Correction may be made for contributions from food AGEs – see below.

Measurement of AGEs for assessment of risk of development and progression of CKD

In a large cross-sectional study, increased serum CML measured by a competitive immunoassay was associated with CKD and negatively associated with GFR.[23] The immunoassay used measured both serum protein CML residues and CML free adduct concurrently.[24] CML free adduct is most sensitive to loss of renal clearance[8] and so such immunoassay formats may be reflecting accumulation of CML free adduct. Total plasma AGEs, the sum protein residues and free adducts, the sum of AGE residues of protein and AGE free adduct, of selected types are determined by analysis of acid hydrolysates of plasma. Total plasma CML and CEL were increased in patients with type 1 diabetes and decreased GFR, compared to those with normal GFR, and were linked to markers of endothelial cell dysfunction - von Willebrand factor, soluble vascular cellular adhesion molecule-1 (sVCAM1), and soluble thrombomodulin – independent of GFR.[25] Total plasma pentosidine was a risk predictor of mortality in CKD after adjusting for all confounders.[26] In recent studies of AGEs and related analysis of skin collagen in patients with type 1 diabetes, an analyte panel of glucosepane, MG-H1, CML, N_{ε}(1-carboxyethyl)lysine (CEL), glyoxal-derived hydroimidazolone (G-H1), pentosidine, furosine, collagen fluorescence, skin collagen acid solubility and pepsin digestibility were linked to risk of progression of diabetic nephropathy; with the FL-linked analyte furosine being the strongest predictor.[27] In contrast, CML residue content of plasma protein was not linked to the risk of developing diabetic nephropathy.[28] Plasma MG-H1 free adduct concentration was an independent risk predictor for progression of DKD.[29] Low molecular weight AGE fluorophore measurement was a mortality predictor in HD patients.[30]

Skin autofluorescence (SAF) has been proposed as an non-invasive measure linked to the dermal content of AGEs.[31] Spectrofluorometric analysis and scanning confocal microscopy and multi-photon excitation microscopy found that major contributions to SAF are from NAD(P)H, flavin adenine dinucleotide and porphyrins.[32, 33] There are also contributions from the oxidative fluorophore, N-formylkynurenine, and trace fluorescent AGEs such as pentosidine and others.[31] Weaknesses of this approach are that SAF has important non-AGE contributions and the major quantitative AGEs in CKD, MG-H1 and CML, are not fluorescent. Change in SAF is likely reflecting a combination of metabolic dysfunction and protein-derived fluorophores in CKD. In assessment of risk of progression of CKD, an optimum cut-off level of SAF gave sensitivity 0.74 and specificity of 0.73 for CKD progression[34] and SAF was also linked to mortality risk in renal failure.[35]

Revisited and new and concepts in AGE-related pathogenesis

The AGE receptor hypothesis revisited

It was proposed that AGE-modified proteins bind specifically to cell surface receptors to activate cell dysfunction. This was questioned and considered limited to proteins structurally-damaged and/or glycated to high, supraphysiological extents prepared *in vitro*.[36, 37] An AGE receptor found influential on the development of experimental diabetic nephropathy and DKD is the receptor for advanced glycation endproducts, RAGE. Transgenic mice overexpressing RAGE showed glomerular hypertrophy, increased albuminuria, mesangial expansion, advanced glomerulosclerosis, and increased serum creatinine compared to non-transgenic diabetic littermates.[38] RAGE-deficient mice show decreased albuminuria, hyperfiltration and glomerulosclerosis compared with diabetic wild-type controls.[39] RAGE expression is increased in peripheral blood monocytes in clinical CKD.[40]

The use of albumin highly glycated by AGEs, dissimilar from albumin which is minimally modified by AGEs *in vivo*, has made studies of the metabolism and functional responses induced by AGE-modified proteins by RAGE and other putative AGE receptors difficult to understand and interpret. Typical glucose-derived AGE-modified albumin prepared *in vitro* had *ca.* 7,000 Da mass increment of glycation adducts, *ca.* 40 – 50 molar equivalents of modification, markedly increase negative charge, bound to RAGE and had rapid clearance by scavenger receptor-mediated removal from circulation in the liver.[41-43] In contrast, albumin *in vivo* typically has <1% modification by one AGE residue, a mass increment of <200 Da (mostly due to early glycation adduct FL), little change in charge, may not always bind RAGE and shows little extraction from circulation in the liver.[13, 22, 41, 44-46] Mathematical modelling of glycation kinetics and protein turnover supports experimental findings of low extents of protein glycation *in vivo*.[22, 47] Protein glycation may reach it highest extent of modification for long-lived basement membrane proteins of the peritoneal cavity basement membrane for renal failure patients receiving long term treatment with PD fluids containing high concentrations of glucose osmolyte. Since RAGE activation is not limited to these conditions, it is likely that ligands other than highly glycated proteins are important agonists for the RAGE receptor physiologically.

Ligand binding studies of RAGE have often been performed with albumin highly modified with AGEs where competitive binding and deductions from crystallographic data of RAGE suggest interaction is driven by the high negative charge of this ligand[48, 49]. There are doubts, therefore, if such interaction is found *in vivo*. Further studies characterised the binding affinities of major AGE residues and AGE free adducts to RAGE by measuring changes of intrinsic tryptophan fluorescence and high resolution nuclear magnetic resonance (NMR) chemical shifts of peptide backbone of the extracellular domain of RAGE.[50, 51] Herein binding affinities were used to deduce percent RAGE occupancy by AGEs *in vivo*. CML and CEL free adducts did not bind to RAGE; and CML and CEL residues had very low affinities

for RAGE such that predicted receptor occupancies in healthy controls and HD patients *in vivo* are very low; only 3 – 8%. This is contrary to initial findings which involved use of CML-modified albumin prepared by chemical reductive alkylation of albumin.[52] Chemically generated CML-modified albumin may have damaged albumin structurally, producing anomalous aggregates which bound to RAGE. In contrast, MG-H1 and related structural isomers, residues and free adducts, have *ca.* 2000-fold higher affinity for RAGE, suggesting MG-derived hydroimidazolone is recognised specifically[50] – Table 1. Albumin modified minimally by MG containing mainly MG-H1 residues was found previously to bind RAGE.[46] The unexpected finding, however, is that RAGE is always saturated with MG-H1 modified protein in healthy subjects and CKD patients, suggesting that this binding may is non-productive for activation of signal transduction. RAGE was recently found to have a high affinity homophilic binding with K_D of 470 nM mediating cell-cell interaction. Hence, normally much of the cellular RAGE protein pool may not be available for AGE-modified protein binding and competent to activate related signal transduction.[53]

Heparan sulfate-RAGE complexes were found to be essential for signal transduction of RAGE activated by AGE-modified albumin, high mobility group protein 1 (HMGB1) and S100 proteins.[54] Signalling downstream from RAGE-heparan sulfate-activating ligand ternary complex studied to date involved extracellular regulated kinase-1 and -2 and p38 MAP kinase phosphorylations. Erk activation mediates pro-fibrotic responses and extracellular matrix expansion.[55] p38 MAP kinase activation is linked to endothelial permeability, inflammation and renal fibrosis.[56-58] Interplay of heparan sulfate and heparanase in CKD may influence the signalling competence of MG-H1 with RAGE.[59] Down regulation of RAGE expression may have beneficial responses, independent of the activating ligand, by countering the increased expression of CKD.[60] Decreased activity of RAGE is associated with decreased podocyte production of monocyte chemoattractant protein-1 (MCP-1),[61] decreased monocyte recruitment, glomerulosclerosis, extracellular matrix accumulation, albuminuria and decline in renal function.[62-64]

Dicarbonyl proteome

Protein dysfunction and inactivation is produced directly by formation of MG-H1 and similar hydroimidazolone AGEs on functional arginine residues of proteins. This likely makes a major contribute to AGE pathogenesis in CKD because: (i) MG-H1 is the AGE of highest flux of formation in CKD; (ii) MG-H1 formation produces loss of charge and all electrostatic interactions of arginine residue modification, eliminating functional interactions and activities; (iii) arginine residues have a high probability of location in functional domains of proteins (20%); and (iv) MG modification tends to occur on functionally-important arginine residues. Proteins modified by MG and related dicarbonyl metabolites are called collectively the "dicarbonyl proteome". Examples of protein targets of MG glycation are: collagen-4 – preferentially modified by MG at integrin binding sites driving endothelial cell detachment, increased circulating endothelial cells and vascular damage;[65] mitochondrial proteins – leading to increased formation of reactive oxygen species;[66] LDL - inducing pro-atherogenic transformation to small dense LDL driving dyslipidemia;[67] p65 of the NF-kB system - leading to increased expression of RAGE and S100A8, S100A12, and HMGB1 and enhanced and persistent vascular inflammation;[68] and apolipoprotein-A1 - inducing de-stabilisation of HDL, contributing to dyslipidemia.[69] Effects of the dicarbonyl proteomes may be modelled *in vitro* by silencing of Glo1. In endothelial cells this triggered increased expression of collagens-1 and -5, endothelin-1, ICAM-1, VCAM-1 and of MCP-1;[70] and in L6 myoblasts increased collagen-1 and -4.[71] This suggests that dicarbonyl stress is a driver of vascular renal inflammation and renal and muscle fibrosis – considered critical to CKD progression and co-morbidities - cardiovascular disease and muscle wasting.[72, 73] The dicarbonyl proteome may

be characterised by high resolution mass spectrometry of tryptic digests of cell protein extracts.[74] This remains to be applied to renal cells and tissue extracts.

Contribution of food AGEs to clinical AGE exposure and health impairment

There is a high content of AGEs in sugar-rich, thermally processed foods. The clinical impact of dietary-derived AGEs remains uncertain. It may be particularly important in CKD where clearance and excretion of AGEs is impaired. Advance on this has been stymied by lack of experimental evidence on the amount of AGEs absorbed from food. Dietary AGEs have low bioavailability. An approach to resolve these is to exploit the measurement of pyrraline – an AGE found exclusively in food[75, 76] – Figure 3. Urinary excretion of AGE free adducts provides an estimate of total body AGE exposure and urinary exertion of pyrraline reflects absorption of AGEs from food. Correlation of urinary excretion of a particular AGE with urinary exertion of pyrraline reflects a significant contribution of the AGE from food. Linear regression of urinary AGE excretion on urinary exertion of pyrraline and extrapolation to zero pyrraline excretion gives an estimate of the flux of AGE formed endogenously in the body. AGE absorbed from food then equals the total urinary AGE flux minus this deduced endogenous AGE flux.[77] This analysis was performed recently for MG-H1. In healthy overweight and obese subjects, the flux of endogenous formation of MG-H1 at baseline was *ca.* 13 nmol/mg creatinine, representing 68% of total MG-H1 exposure; the mean contribution to total MG-H1 exposure from the diet was 32% but highly variable.[77] Similar studies are now required in CKD patients. The diet is likely often a minor source of MG-H1 exposure.

Several clinical studies with interventions to decrease exposure to dietary AGEs in CKD for improved vascular health have been performed, with often assessment of markers of vascular inflammation.[78, 79] These and other studies were evaluated by meta-analysis for overall assessment of evidence of health benefits.[80] There are difficulties of interpretation as unblinded study designs were used. It was concluded that there is insufficient evidence, at present, of health benefit to recommend dietary AGE restriction in patients with CKD.[80] An advance that was thought would improve studies of this type was direct determination of the proportions of AGEs exposure originating from the diet endogenous origin. This has recently been provided[77] – see above. There is a need for long-term, high-quality randomised controlled trials with large sample size and masking of the AGE intervention to provide more robust evidence on the health impact of dietary AGEs in CKD – also taking advantage of recent advances in improved quantitation of AGEs in food.[81] In preclinical studies, it was found that diabetic mice on a low AGE diet still developed diabetic nephropathy.[82] A recent open label, pilot study found effects of a high AGE diet on intestinal microbiota in renal failure patients receiving PD therapy.[83]

AGEs formed endogenously in proteins are likely the most damaging to physiological function. Dietary AGEs are absorbed as free adducts after digestion or as peptides which are hydrolysed by peptidases to AGE free adducts after absorption.[22] To date, there is no evidence that AGE free adducts from food may be incorporated into endogenous proteins but they may equilibrate with pools of AGE free adducts in plasma and tissues. Endogenous flux of formation of MG-H1 and other AGEs may be improved AGE-related clinical biomarkers to assess association with progression of CKD and risk of associated co-morbidities.

AGE-related therapeutics - Glyoxalase 1 inducers

Glycation by dicarbonyls is non-oxidative and so it is insensitive to antioxidants. It requires a new type of pharmacological agent for therapy. Experimental agents have been developed to scavenge reactive dicarbonyls and an existing therapeutic agent for patients with type 2 diabetes (T2DM), metformin, also reacts with MG. These provide ineffective removal of MG

compared to *in situ* activity of Glo1 – Table 2. Decrease of MG levels in patients with T2DM treated with metformin[84] is likely mainly achieved through increase in glycaemic control.[85] Therapeutic agents that induce Glo1 expression may be better because: (i) Glo1 metabolises >97% of MG formed in human metabolism;[10] (ii) they correct decreased renal Glo1 activity which is a common characteristic of animal models of DKD;[86-88] and (iii) pre-clinical studies showing decreased Glo1 expression potentiates and overexpression of Glo1 prevents the development of diabetic nephropathy.[7, 12, 89]

Small molecule inducers of Glo1 expression or "Glo1 inducers" may be developed as activators of Nrf2, exploiting a regulatory antioxidant response element (ARE) in the GLO1 gene. Glo1 inducers decreased cellular and extracellular concentrations of MG, MG-derived AGE formation and related functional impairment - such as endothelial attachment to collagen-4.[90] We screened Glo1 inducers with a functional assay based on GLO1-ARE linked expression.[90] The best Glo1 inducer was a combination of *trans*-resveratrol (tRES) and hesperetin (HESP), tRES-HESP.[77]

tRES-HESP combination was evaluated in Phase 1 clinical trial in overweight and obese subjects to establish safety and target pharmacology. Functional assessments of metabolic health were also included - Phase 2A trial for metabolic health benefit in obesity. The study was a randomized, double blind, placebo-controlled crossover study in 29 subjects. Dosing was by oral capsule, once daily, containing tRES-HESP or placebo. Dosing periods were 8 weeks with 6 weeks washout between in the crossover period. Urinary excretion of tRES and HESP metabolites was increased >2000-fold and >100 fold, respectively, compared to placebo. Clinical safety indicators were normal at study entry and remained unchanged throughout the placebo and tRES-HESP treatment periods. tRES-HESP treatment produced a 22% increase in Glo1 activity of peripheral blood mononuclear cells (PBMCs) of all subjects, compared to placebo. The increase was 30% in the obese sub-group (subjects with BMI $\geq$ 30 kg/m^2). Concomitant with increased Glo1 activity, there was a 37% decrease in plasma MG post-supplementation with tRES-HESP but not with placebo. The flux of endogenously-generated MG-H1 adducts was *ca.* 13 nmol/mg creatinine at baseline and decreased by 14% with tRES-HESP treatment but not with placebo.[77]

tRES-HESP treatment produced a profound effect on glucose metabolism. Insulin resistance was corrected to levels typical of lean subjects with normal insulin sensitivity; dysglycaemia was improved - fasting plasma glucose decreased 5% and 2-h area-under-the-curve plasma glucose in an oral glucose tolerance test decreased 8%. This suggests tRES-HESP may be highly beneficial for treatment of insulin resistance in CKD. Other changes were: 3% increase in eGFR and 9% decrease in plasma urea with tRES-HESP. The study participants had stages G1 and G2 CKD with renal function assessments were made at only once at study visits and so these responses require validation in further studies and advanced stages of CKD.

There was also a profound effect of tRES-HESP on inflammatory gene expression, assessed in PBMCs. In all subjects there was a 30% decrease of prostaglandin-endoperoxide synthase 2 (PTGS2 or COX2) and 39% decrease of interleukin-8 (IL-8). In the highly-overweight subgroup there was also marked decreases of RAGE (37%) and MCP-1 (49%). Treatment with placebo produced no change. Inhibition of RAGE and MCP-1 signalling are targets for drug development in CKD.

The health beneficial effects of tRES-HESP have not been achieved by these compounds individually, even at markedly higher doses.[91, 92] The likely explanation for this is synergistic pharmacological effects in activation of Nrf2[77] and improved bioavailability of tRES through inhibition of intestinal glucuronosyltransferases by HESP.[93] If confirmed, this circumvention of bioavailability problems of tRES may be a major advance to translating promise of health benefits of tRES for clinical applications – Figure 5. In experimental models of DKD, tRES

and hesperidin – a glycoside derivative of HESP, decreased albuminuria, glomerular matrix expansion, inflammation and renal function decline, albeit at doses that are not translatable clinically.[94, 95]

The Glo1 inducer appears to be particularly appropriate and timely for treatment of CKD, addressing multiple targets of cell and tissue dysfunction. It may correct renal extracellular matrix disposition and dysfunction, fibrosis and inflammation – allowing for re-engagement of podocyte food processes and re-establishment of normal glomerular endothelial cell, mesangial cell and fibular cell function to re-instate the glomerular filtration barrier and improve GFR – Figure 4. Some of the benefits of tRES-HESP may be due to concurrent induction of increased expression of other protective ARE-linked genes - including protection against other dicarbonyls since expression of AKRs was also induced.[77]

Concluding remarks

It is likely that dicarbonyl stress and increased formation of MG-H1 is a key contributory factor in the development of CKD for which down regulation of renal Glo1 appears to be a key driver. Correction of this by Glo1 inducers offers a new strategy for treatment and measurement of flux of MG-H1 new companion diagnostics to guide their use in precision medicine. New features of AGE research in CKD are summarised in Table 3.

ACKNOWLEDGEMENTS

We thank our research team and collaborators for their efforts on MG and Glo1-related research.

DISCLOSURE

The authors are inventors on a patent of the Glo1 inducer tRES-HESP and related formulations.

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Table 1 | Binding affinities and predicted *in vivo* occupancy of RAGE with AGE residues and AGE free adducts.

Ligand	K_D (μM)	[AGE], healthy control and stage 5 CKD (μM)	Receptor occupancy in healthy subjects (%)	Receptor occupancy in HD patients (%)
CML residue	87	2.3 and 8.0	2.6	8.4
CEL residue	93	1.1 and 2.6	1.2	2.7
MG-H1 residue	0.040	12.8 and 22.0	99.7	99.8
MG-H1 free adduct	0.040	0.12 and 4.8	54.9	99.0
G-H1 free adduct	0.043	0.04 and 0.26	21.6	32.7
RAGE	0.47	0.13 and 0.73	18.4	45.7

Assumptions: RAGE protein copy number in human monocytes *in vivo* was 37,000 and 208,000 per cell in healthy subjects and HD patients, respectively,[40] equivalent to a concentration of *ca.* 130 and 730 nM. K_D and AGE concentration values as given.[13, 50, 51] Receptor occupancy was determined by deducing the equilibrium position of the binding equation AGE + RAGE = AGE-RAGE.

Table 2 | Experimental therapeutic agents to counter dicarbonyl stress.

Agents	Mechanism of action	Advantages/advantages
Glo1 inducer	Increased expression of Glo1 (high efficiency catalytic removal of MG and glyoxal)	Corrects deficiency of Glo1 in the kidney and elsewhere driving dicarbonyl stress-induced mechanisms of CKD.[88] Limited to effects on Glo1 substrates. Induction occurs rapidly within a few hours and is relatively long lasting; Glo1 half-life is 2 – 3 days.[77, 90]
Metformin	Dicarbonyl scavenger (stoichiometric removal)	Known pharmacological and safety profile. Reacts with MG to form mainly hydroimidazolone-like and also triazepinone adducts but they are quantitatively minor compared to the flux of MG formation.[96, 97]
Aminoguanidine (Pimagedine)	Dicarbonyl scavenger (stoichiometric removal)	Aminoguanidine reacts with MG and other dicarbonyls to form 3-amino-1,2,4-triazine derivatives.[98, 99] At peak concentration, aminoguanidine is an effective MG scavenger in plasma but not inside cells where it is expected to compete ineffectively with Glo1 but this is short-lived; plasma half-life is *ca.* 1.4 h. [100] In clinical trial adverse effects were gastrointestinal disturbance, abnormal liver function tests, flu-like symptoms and a rare vasculitis.[101] Clinical therapeutic development was questioned further when kidney tumours were found in aminoguanidine-treated diabetic rats.[102]
Phenacylthiazolium bromide (PTB) and related derivatives e.g. Alagebrium	Dicarbonyl scavenger (stoichiometric removal)	PTB is an efficient scavenger of MG*; ca.* 6-fold more effective than aminoguanidine under physiological conditions.[98, 103] Unstable under physiological conditions, degrading by hydrolysis with a half-life of 44 min.[104] An effective MG scavenger in plasma for a short period but not inside cells where it is expected to compete ineffectively with Glo1. Alagebrium shows similar properties.[105] It improved albuminuria in experimental diabetic nephropathy.[106] A Phase 2 clinical trial in patients with type 1 diabetes and microalbuminuria was terminated early for financial reasons (identifier NCT00557518, clinicaltrials.gov).
Pyridoxamine	Dicarbonyl scavenger (stoichiometric removal)	Well tolerated. Relatively low reactivity with dicarbonyls.[107] May function through other, non-AGE mechanisms (supplementation of vitamin B_6 metabolism).[108] Judged to have failed in phase 2 clinical trial for treatment of diabetic nephropathy.[109]

Table 2 | Experimental therapeutic agents to counter dicarbonyl stress (cont'd).

Agents	Mechanism of action	Advantages/advantages
Arginine and arginine-rich peptides	Dicarbonyl scavenger (stoichiometric removal)	Well tolerated but poor scavenger activity: median effective concentration of arginine in an *ex vivo* model of plasma protein glycation was 23 mM.[110] These agents are competing with high endogenous cellular and extracellular arginine residue concentrations; *ca.* 20 mM and 80 mM, respectively. Improved MG scavenging was found with arg-arg and arg-lys residue containing peptides.[86, 111]
TTP488 (PF-04494700)	RAGE inhibitor	May block RAGE signalling.[112] It was evaluated in Phase 2 clinical trial for DKD (6 month treatment, 110 patients). It failed to show effect on urinary albumin-creatinine ratio, eGFR or potential markers of RAGE inhibition.[113]

Table 3 | New features of AGEs in CKD.

Feature	Description	Key evidence	Reference
Dicarbonyl stress	Dicarbonyl stress is a driver of CKD development which is exacerbated as CKD progresses.	Glo1 silencing in mice imposes a nephropathy phenotype. Experimental and clinical advanced CKD exhibit dicarbonyl stress.	5, 7, 8
MG accumulation is of endogenous origin	MG accumulation is driven by down regulation of Glo1 in CKD. Endogenous flux of MG formation far exceeds MG of exogenous origin	Expression and activity of Glo1 is down regulated in experimental CKD. D-Lactate concentration is not increased in CKD.	9, 10
MG-H1 is a major challenge to proteostasis in CKD	MG-H1 formation impairs protein function and increased in CKD	Dicarbonyl proteome and flux of MG-H1 increased 9-fold in CKD; 4 – 713 fold higher flux that other AGEs.	13, 67
AGE/RAGE interaction	Functional activity of AGEs with RAGE is uncertain	CML and CEL residues have too low affinities to bind RAGE *in vivo*; MG-H1 binds RAGE but binding is always saturated	13, 50, 51
Dietary AGEs	The amount of AGEs absorbed from the diet may be deduced from urinary AGE and pyrraline fluxes.	Regression of urinary MG-H1 excretion on urinary pyrraline excretion.	77
Glo1 inducer therapeutics	Small molecule inducers of Glo1 expression to counter down regulation of renal Glo1 in CKD.	tRES-HESP combination evaluated in Phase 1/Phase 2A (obesity) clinical trial.	77

FIGURE LEGENDS

Figure 1 | Major AGEs in chronic kidney disease.

Figure 2 | The glyoxalase system. Schematic of the glyoxalase metabolic pathway.[10]

Figure 3 | Pyrraline – an AGE exclusively of dietary origin.

Figure 4 | Expected mechanism of action of glyoxalase 1 inducers in chronic kidney disease. Evidence form studies of Glo1 functional genomics and Glo1 overexpression, Glo1 inhibitor and MG treatment of renal cells *in vitro*. Abbreviations: SELE, E-selectin; VCAM-1, vascular cell adhesion molecule-1; Tie2, tyrosine-protein kinase cell-surface receptor for angiopoietin-1, angiopoietin-2 and angiopoietin-4; VEGF, vascular endothelial growth factor; TGFbeta, transforming growth factor-beta.

Figure 5 | Circumventing the problem of low bioavailability of *trans*-resveratrol Impairment of glucuronidation of *trans*-resveratrol and hesperetin in the small intestine by co-administration at pharmaceutical doses. Comparison of the demands on glucuronidation in the intestinal epithelium when tRES alone or in combination with HESP are administered. Abbreviations: HESP-3'-O-G, hesperetin-3'-O-glucuronide; HESP-7-O-G, hesperetin-7-O-glucuronide; tRES-3-O-G, trans-resveratrol-3-O-glucuronide; tRES-4'-O-G, trans-resveratrol-4'-O-glucuronide.

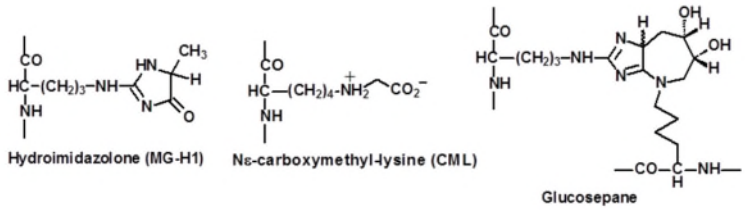

Figure 1

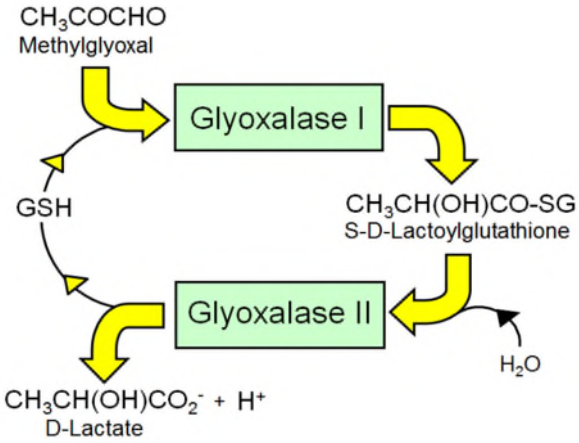

Figure 2

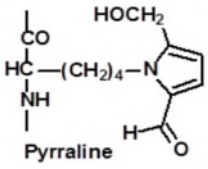

Figure 3

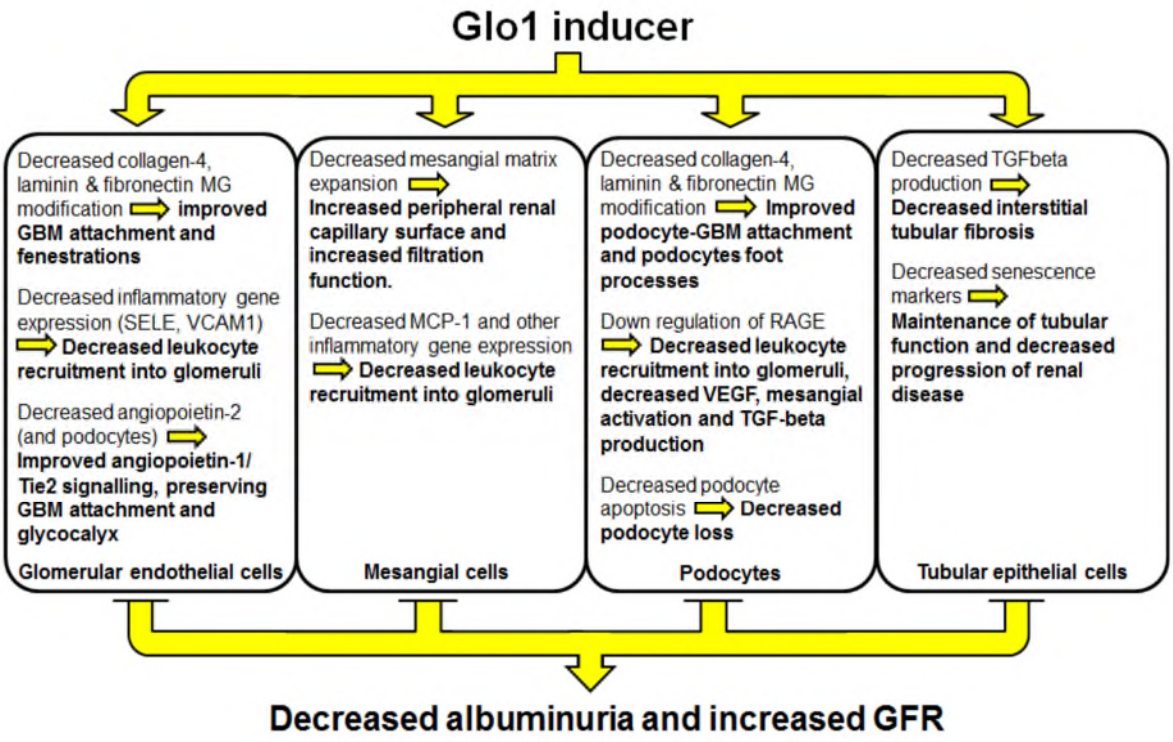

Figure 4

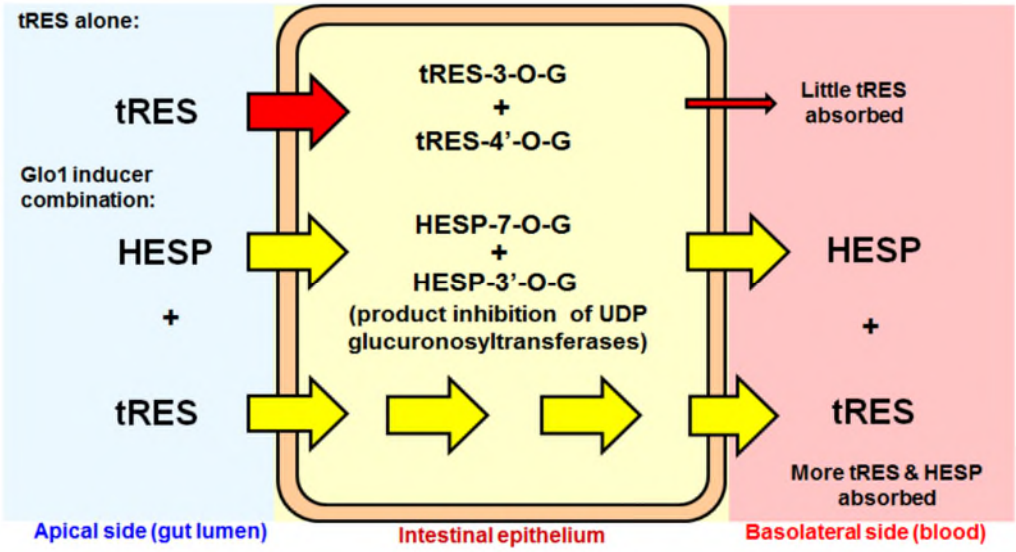

Figure 5