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Identifying Advanced Glycation End Products as a Major Source of Oxidants in Aging: Implications for the Management and/or Prevention of Reduced Renal Function in Elderly Persons

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Summary

Aging is characterized by increasing inflammation and oxidant stress (OS). Reduced renal function was present in more than 20% of normal-aged individuals sampled in the National Health and Nutrition Examination Survey (NHANES) cross-sectional study of the US population. Longitudinal studies in the United States and Italy showed that renal function does not decline in some individuals, suggesting that a search for causes of the loss of renal function in some persons might be indicated and interventions to reduce this outcome should be sought. Because advanced glycation end products (AGEs) induce both inflammation and OS, accumulate with age, and primarily are excreted by the kidney, one outcome of reduced renal function in aging could be decreased AGE disposal. The build-up of AGEs with reduced renal function could contribute to inflammation, increased oxidant stress, and accumulation of AGEs in aging. In fact, results from a longitudinal study of normal aging adults in Italy showed that the most significant correlation with mortality was the level of renal function. A clear link between inflammation, OS, AGEs, and chronic disease was shown in studies of mice that showed that reduction of AGE levels by drugs or decreased intake of AGEs reduces chronic kidney disease (CKD) and cardiovascular disease of aging. The data support a role for AGEs in the development of renal lesions in aging mice and reveal that AGEs in the diet are very important contributors to renal and cardiovascular lesions. AGEs signal through two receptors, one of which is anti-inflammatory (AGER1) and the other is proinflammatory (RAGE). Overexpression of AGER1 protects against OS and acute vascular injury. The reduction of AGEs in the diet is as efficient in preventing aging-related cardiovascular and renal lesions in mice as that seen with calorie restriction. Studies in normal adults of all ages and those with CKD suggest that the findings in mice may be directly applicable to both aging and CKD. Namely, the dietary content of AGEs determines the serum levels of AGEs and

inflammatory mediators and urine AGE levels in both normal subjects and CKD patients. Importantly, reduction of AGEs controls these changes in both normal subjects and CKD patients, and the phenotypic changes in AGER1 are reduced in CKD patients by decreasing the amount of AGEs consumed with the diet. These data suggest that the changes in renal function in normal aging may be subject to control and this subject deserves renewed attention.

Keywords

AGEs; aging; diet; CKD

Twenty million adults in the United States (age, 20 y) have an increased serum creatinine level and the incidence increases with age according to a large cross-sectional study (National Health and Nutrition Examination Survey [NHANES III]).¹ More than 20% of those age 65 years or older have decreased renal function, even when corrected for obvious causes of renal diseases such as hypertension and diabetes mellitus. Thus, de novo chronic kidney disease (CKD) of aging represents an important health problem. Hypertension and diabetes mellitus, both of which are associated with increased oxidative stress (OS), and intercurrent events that increase OS carry a further increase in the risk of renal failure.¹ This suggests that the aging kidney is more susceptible to injury and/or has an altered repair response.² There is a strong association between CKD and cardiovascular disease, both of which have been associated with an inflammatory state.³⁻¹¹ These data are consistent with our observations in experimental animals and human beings, that the presence of adequate renal function is critical to maintaining the body load of AGEs, at nontoxic levels.¹² Namely, serum advanced glycation end products (AGEs) correlate directly with the levels of inflammatory markers and OS, and inversely with the clearance of creatinine.¹³ CKD in aging also is associated with increased levels of interleukin-6, C-reactive protein, and other markers of inflammation.¹⁴ Thus, the CKD of aging is associated with an inflammatory state, which may be associated with the general increase in OS noted with aging. CKD that resembles that seen in human beings occurs in multiple mouse strains and in rats and consists of glomerulosclerosis and hypertrophy, tubulointerstitial changes, chronic inflammation, and vascular lesions.^{15,16}

AGEs

These compounds are generated endogenously when reducing sugars, ascorbate, and other carbohydrates spontaneously react with amino-peptides, nucleic acids, and amine-containing lipids.^{17,18} The diet is an underappreciated source of AGEs.^{19,20} This exogenous source consists of foods cooked at increased temperatures.²¹ Amine-containing lipids form advanced lipoxidation end products such as 4-hydroxynonenal, or ^εN-carboxymethyllysine (CML) and their analogues, deriving from both lipid and protein oxidation.^{22,23} Glycoxidation of proteins and lipids (AGEs/advanced lipoxidation end products) involves the formation of reactive carbonyls that generate reactive oxygen species (ROS) and increased OS, termed *carbonyl stress*.^{24,25} Autooxidation of glucose is accompanied by the generation of ROS such as superoxide radicals.²⁴ ROS enhance glycation, and both

mechanisms can interfere with the normal clearance of macromolecules (ie, low-density lipoprotein), from circulation and promote atherogenesis.^{22,23,26}

The term *AGEs*, although often referring to nonreactive terminal products, such as CML or pentosidine, also includes reactive precursors, such as methylglyoxal, glyoxal, and 1- or 3-deoxyglucosone. Methylglyoxal constantly is produced in vivo by glucose auto-oxidation and intermediates of protein and lipid metabolism. Glyoxalase I is a major methylglyoxal detoxifying enzyme. The action of glyoxalase I requires glutathione. Glutathione reacts nonenzymatically with methylglyoxal to form S-D-lactoylglutathione hemithioacetal. Glyoxalase I breaks hemithioacetal adduct to S-D-lactoylglutathioneion. Methylglyoxal-derived AGEs (MG) are one of the major groups of AGEs. MG is generated primarily from the interaction of methylglyoxal with arginine residues. MG is more active because of its dynamic reversibility. The levels of MG decrease when the concentration of methylglyoxal decreases. AGEs including MG levels are increased in diabetes and aging.^{27,28} The cause of increased AGEs in aging is not clear. Because the kidney is the major site for the excretion of AGEs, a mild decrease in renal function may be associated with increased levels of AGEs. The accumulation of AGEs with the slowly progressive decrease in renal function in aging may induce a release of inflammatory mediators and the generation of ROS before a clinically evident decrease in kidney function becomes evident.^{12,29} Chronic inflammation and oxidative stress are risk factors for kidney aging. The significance of AGE accumulation in aging is established by the finding that AGE inhibitors conferred significant protection against cardiovascular disease and CKD in aging.³⁰⁻³²

AGE LEVELS AND RENAL FUNCTION

AGEs are excreted by the kidney, and their levels are increased in patients with renal insufficiency, with or without diabetes.^{33,34} Although it has long been known that the levels of circulating AGEs are increased in patients with already developed renal failure, it now is clear that AGEs are increased before there is clinical evidence of reduced renal function^{13,19,29} in both the normal population and in diabetic patients.^{12,20} The kidney is both the only major site for excretion of terminal end products of AGE metabolism and a target for reactive early AGE intermediates. Reactive intermediates attached to small peptides may be filtered by the glomerulus and taken up in the proximal tubules from the filtrate or they may be directly taken out of the blood in the peritubular capillaries. The proximal tubule may further metabolize AGEs to nonreactive products. Thus, the kidney is one of the key regulators of AGE metabolism in aging. However, the ingested AGEs may induce an inflammatory response in the tubules. This being the case, it is critical to understand the pathways by which AGEs mediate inflammatory responses in the kidney.

AGEs AND AGE-RELATED RECEPTORS

AGEs bind to cellular AGE-receptors inducing inflammation and ROS.^{35,36} The AGE receptor system is complex, and includes RAGE, AGER1-3, ScR-II, CD36, and defensins.³⁷ Some receptors remove excess AGE from the body (AGER1) and others increase OS and activate inflammatory responses (eg, RAGE).^{38,39} Those that activate an inflammatory response have been studied extensively, and are best represented by a multiligand protein,

RAGE, which is linked to OS and cell activation.^{37,40} Although insight has been obtained from in vitro molecular studies on certain AGE receptors, clarification of their in vivo role has been difficult. For instance, reactive AGEs affect cells and cellular AGE receptors alike; RAGE, the receptor most closely associated with ROS and nuclear factor- κ B activation, also is inducible by AGE and ROS.³⁸ Despite high expression/function of RAGE in diabetes, renal disease, and aging, tissue AGE deposits remain increased, suggesting that it does not participate in AGE removal. AGER1, which clears AGEs, appears to be suppressed or down-regulated in the presence of excess AGE-induced oxidant stress (ie, severe diabetic complications and aging).^{28,41} We found that AGER1 could have a protective function against OS because it may inhibit AGE-dependent cellular activation.

STUDIES IN MICE

Characterization of the Kidney Lesions in Aging C57B6 Mice

Glomerular changes in C57B6 mice begin at 18 to 20 months of age, before recognizable tubulointerstitial changes, and progressively increased to death at 30 months.⁴²⁻⁴⁴ These mice develop albuminuria and decreased renal function. We also found that AGEs increase in the kidney with aging,²⁸ and that a reduction of the intake of AGEs via the diet blocked the development of the CKD of aging and prolonged survival. We found that glomerular mesangial cells isolated from aging mice express stable phenotypic changes, including increased production of inflammatory cytokines and connective tissue proteins, oxidant stress, cellular hypertrophy, and increased P27 levels.^{43,44} Because a proliferative stimulus accentuates kidney lesions, we reasoned that uninephrectomy would accelerate the CKD of aging. Uninephrectomy in 20-month-old C57B6 mice resulted in accelerated kidney lesions (glomerular and tubulointerstitial), increased albuminuria, and early onset renal failure.⁴² The lesions closely resembled those found in 30-month-old mice, suggesting the added proliferative stimulus from a reduction in nephron number accelerated the aging changes and that phenotypically altered recruited progenitors could be the source of the inflammatory/prosclerotic changes.

Determination of the Anatomic Site of the Phenotypic Change

We previously observed that the bone marrow contained mesenchymal progenitors in human beings and glomerular progenitors in mice,⁴⁵ and that the bone marrow contains progenitors that express stable phenotypic changes in glomerulosclerosis and diabetes. Because the bone marrow contained altered progenitors in other chronic renal diseases, we postulated that the lesions in the CKD of aging also may originate in a progenitor population. Therefore, we asked whether the bone marrow is a source of kidney progenitors that carry the inflammatory/prosclerotic phenotypic changes of aging. We found the following.

The bone marrow contains glomerular progenitors that express the inflammatory/sclerosis phenotype. Bone marrow transplants from early and late aging mice transfer both the glomerular and tubulointerstitial lesions found in aging donors to young naive recipients. Thus, bone marrow-derived progenitors mediate at least some aspects of the aging lesions in C57B6 mice.⁴⁴ The lesions transferred were donor age-specific. Young recipients of bone marrow transplants from the oldest donors had more severe lesions than if the donor was less

old. This suggests that the number of progenitors in the bone marrow, which express stable phenotypic changes, increase with age. These aspects apply to aging in both human beings and mice.

Bone marrow transplants from young donors largely reversed kidney lesions in old recipients. Note that there was a decrease in glomerular size, sclerosis, and cellularity, coupled with a decrease in tubulointerstitial lesions. Thus, the glomerular and tubulointerstitial lesions of aging are reversible in C57B6 mice, and progenitors derived from the bone marrow mediate these changes. These data confirm that bone marrow-derived progenitors may play an active role in the CKD of aging.⁴⁴ The data also suggest that delivery of normal progenitors reduces the CKD of aging, in an aging environment.

Stable phenotypic changes in kidney-specific progenitors occur in a number of chronic sclerosing/inflammatory conditions, and are not limited to a specific genetic background. Transfer of progressive sclerosing kidney lesions by bone marrow-derived progenitors also was found in a radiation-induced mutant mouse strain (ROP^{-/+}) with progressive glomerulosclerosis (G2) and in a model of diabetic nephropathy C57B6/db^{-/-}.^{45,46}

The Molecular Basis of the Stable Phenotypic Change

We found that mesangial cells from aging mice have a stably altered phenotype: mesangial cells isolated from 28-month-old mice show stable phenotypic changes.⁴² In addition, the in vitro phenotype and in vivo phenotype are correlated. Namely, the stable phenotypic changes in mesangial cells in vitro reflect the in vivo sclerotic glomerular changes.^{47,48} The stable aging phenotype is proinflammatory and prosclerotic, as shown by gene expression analysis that revealed that the stable phenotypic changes in glomerular cells were proinflammatory and prosclerotic.⁴³ Although not applicable to aging, per se, stable epigenetic changes in the matrix metalloproteinase-9 promoter could be induced by chronic hyperglycemia in vitro, a condition associated with increased formation of AGEs and increased OS.^{48,49} We found that the reversibility of the phenotypic changes in the matrix metalloproteinase-9 promoter depended on the genetic background.⁴⁹

Amelioration of Phenotypic Changes in Mesangial Cells by Peroxisome Proliferator Activated Receptor- α , Pyridoxamine, and Estrogen

Mice with type 2 diabetes and established nephropathy were treated with peroxisome proliferator activated receptor- α agonists, which improves glucose use, or with pyridoxamine, which reduces AGE formation.⁵⁰⁻⁵² Both drugs reduced renal lesions and improved renal function and favorably affected matrix turnover by mesangial cells in vitro. These data provide evidence that the stable phenotypic changes can be modulated, with resultant stabilization or reversal of established kidney lesions. Mesangial cells express both the α and β subtypes of the estrogen receptor and estradiol administration results in up-regulation of matrix metalloproteinase-2 and -9.⁵³ Estrogen receptors also are decreased at the transcriptional level in ROP^{+/-} mice, which are prone to develop glomerulosclerosis, revealing that glomerulosclerosis in certain mouse strains may be associated with estrogen deficiency, a feature of the CKD in aging.^{42-44,54} The induction of profound estrogen deficiency in ROP^{+/-} mice results in acceleration of the glomerular lesions.⁵⁴ In this model,

we found that the maintenance of normal glomerular function depended on the continuous presence of physiologic levels of estrogen, and that prolonged estrogen deficiency induced stable phenotypic changes in mesangial cells.⁵⁵ Estrogen and progesterone receptors and responses to estrogen and progesterone also were studied in the vascular cells of C3H/HeJ (toll-like receptor [TLR]4-deficient) mice and found that collagen synthesis and degradation were not responsive to added estrogen or progesterone, despite the presence of these receptors.⁵⁶ These data also suggest that both the levels and responses to sex hormones may be important in the development of, and responses to, stable phenotypic inflammatory/sclerotic changes.⁵⁵ They also suggest that the TLR4 deficiency and transforming growth factor- β -mediated responses may be involved, providing a link between AGEs and fibrosis.⁵⁶

Suppression of Inflammation Reduces CKD

The inflammatory aspects of progressive kidney diseases were prevented by pentosan polysulfate, a drug that decreases inflammation in the kidney. Namely, pentosan polysulfate prevented the development of kidney lesions after five-sixths nephrectomy, a model pertinent to CKD of aging.⁵⁷

Decreasing AGE Intake Reduces the CKD of Aging and Decreases AGE Accumulation and Transforming Growth Factor- β 1 and Type IV Collagen Messenger RNA Expression in the Aging Kidney

Aminoguanidine decreases the cardiovascular and renal disease of aging in rats.³⁰ Because dietary AGEs are a major source of oxidants in aging, we introduced a life-long restriction of AGEs in mice to determine if external AGEs served as a source of the AGE accumulation in aging.

Thus, restriction of AGEs in the diet reduces kidney lesions in aging. We also found that the myocardial changes of aging are reduced and longevity is increased by the low-AGE dietary intervention (see later).⁵⁸

Decreasing AGE Intake, Without Decreasing Calorie Intake, Reduces the CKD of Aging and Increases Lifespan

As shown earlier the content of AGEs in the diet correlates with serum AGE levels, OS, organ dysfunction, and lifespan. We also found that the addition of a chemically defined AGE (methylglyoxal-bovine serum albumin) to a low-AGE mouse chow increased levels of serum AGEs and OS (>2- to 3-fold). This provides evidence that dietary AGEs are oxidants that can induce systemic OS, which predisposes to cardiovascular disease and CKD. Calorie restriction (CR) is the most studied means to decrease OS, increase longevity, and reduce OS-related organ damage in mammals. We hypothesized that because reduction of food intake obligatorily also decreases oxidant AGE intake, the beneficial effects of CR in mammals could be related to restriction of oxidants or energy. We studied mice pair-fed a CR diet or a high-AGE CR diet in which the dietary content of AGEs was increased by brief heat treatment (CR-High). We found that old mice given CR with a brief heat treatment developed high levels of 8-isoprostanes, AGEs, RAGE, and p66 shc, coupled with low AGER1, low GSH/GSSG, insulin resistance, marked myocardial and renal fibrosis, and a

shortened lifespan.⁵⁸ Similar to mice maintained on an AGE-restricted diet, old CR mice also had low OS, p66 shc, RAGE, and AGEs, but high AGER1, coupled with a longer lifespan. These data lead us to conclude that the beneficial effects of a CR diet may be related partly to a reduced oxidant intake, and that a reduced intake of oxidant AGEs via the diet is one of the principal determinants of oxidant status in aging mice.

STUDIES IN HUMAN BEINGS

Normal Subjects, Cross-Sectional Data, New York Subjects

We focused on two distinct serum AGEs. First, N^ε-carboxy-methyl-lysine (sCML), a terminal oxidant product, which is less active and likely to reflect longitudinal exposure.⁵⁹ Second, methylglyoxal derivatives (sMG) are more active, less stable, and readily derived from ROS of other sources. The cross-sectional analyses of approximately 300 healthy persons established that levels of sCML and sMG correlate with each other, and both are higher in older than in younger persons and in men compared with women older than age 65.¹⁹ Also, the levels of serum AGEs correlated with circulating inflammatory markers, such as 8-isoprostanes, vascular cell adhesion molecule (VCAM)-1, tumor necrosis factor (TNF)- α , and C-reactive protein. After adjusting for sex, age, and renal clearance (based on estimated glomerular filtration rate), serum AGEs were independent predictors of OS among healthy persons. A pivotal finding during this investigation was that dietary AGE intake, adjusted for calorie and nutrient intake, significantly correlated with levels of sCML, sMG, 8-isoprostanes, VCAM-1, and mononuclear TNF- α , among other inflammatory markers across age. These findings suggested that persons habitually consuming high-AGE diets may have abnormally high levels of AGEs, which may exceed the capacity of native defenses to remove or neutralize them. Thus, independent of age, chronic consumption of AGE-rich foods may cause high OS, a common denominator for most chronic diseases.

Normal Subjects, Longitudinal Data, New York Subjects

Repeated measures of serum AGEs and dietary AGEs at quarterly intervals over a period of 2 years confirmed the earlier-described relationships.¹⁹ Serum CML levels (as well as sMG) varied during this period and the changes significantly correlated with changes in 8-isoprostanes, peripheral mononuclear cell-derived TNF- α , and VCAM-1. Moreover, changes in dietary AGE intake correlated with those of sCML, as well as of sMG. These findings were in line with the baseline cross-sectional data and supported the proposed link between exogenous oxidant load and systemic oxidant and inflammation burden.

Normal Subjects, Longitudinal Data, Baltimore Subjects

The first systematic longitudinal scientific examination of human aging was started in 1958: the Baltimore Longitudinal Study of Aging. This cohort has remained as one of the most important sources of information on the aging kidney.⁶⁰ Data from the Baltimore Longitudinal Study of Aging confirmed that renal function shows a progressive decline of renal function in some normal aging adults (based on age-adjusted standards for creatinine clearance (CrCl)).⁶¹ Based on CrCl the estimated average annual change in CrCl equaled $-0.26 \text{ mL/min/1.73 m}^2$ in individuals aged 20 to 39 years, and $-1.51 \text{ mL/min/1.73 m}^2$ after age 80. These data are unique in that the predictions were based on cross-sectional and

longitudinal observations and only reported for individuals considered healthy based on strict standard criteria. Interestingly, although the longitudinal data confirmed the cross-sectional observations, they also identified two groups that were not apparent in the cross-sectional analysis, one had an accelerated decline of kidney function with age, and another that did not show any loss of renal function with age. Surprisingly, some Baltimore Longitudinal Study of Aging participants did not have a significant decline in renal function over 5 to 10 years. Although the number of these individuals was small and few were older than 70 years of age, these data challenge the notion that the decline of kidney function with age is unavoidable and call for increased investigation in this area. Similar data were reported in a letter to the editor of the *New England Journal of Medicine* by investigators examining living kidney donors, in which an extensive follow-up evaluation failed to show a decline in renal function in this population selected for the absence of kidney disease.⁶²

Normal Subjects, Longitudinal Data, Italian Subjects

Data from another longitudinal study in Italian normal aging subjects (InCHIANTI study) addressed the question of whether measured and estimated CrCl in older individuals are significant predictors of mortality.⁶³ They used different formulae for calculating the glomerular filtration rate and found that after stratifying by CrCl, patients with CrCl levels of less than 60 mL/min/1.73 m² and 60 to 90 mL/min/1.73 m² were more likely to die over the 6-year follow-up period compared with those with CrCl levels of more than 90 mL/min/1.73 m² (hazard ratio, 1.91; 95% confidence interval, 1.11-3.29; and hazard ratio, 1.70; 95% CI, 1.02-2.83, respectively). They did not find that classification based on the Modification of Diet in Renal Disease (MDRD) formula provided significant prognostic information for mortality.

Normal Subjects, Low-AGE Intervention Study

The relationship of exogenous AGEs to that of internal OS state in healthy human beings was approached by a nonpharmacologic intervention, namely an isocaloric low-AGE diet that had been validated previously in patients with diabetes or with CKD.¹⁹ Healthy subjects were assigned randomly to either their own regular diet or a low-AGE diet (AGE levels 50% below the amount consumed normally).⁶⁴ The diets were otherwise of equal caloric, nutrient, and micronutrient content. Subjects were selected on the basis of a habitually high-normal diet AGE intake (>15 AGE Eq/d). Thus, their entry levels of serum AGEs and related markers were also at the upper end of the normal range. Participants received instructions on how to prepare the diet at home. After 4 months, significant reductions in sCML and sMG levels were noted, with parallel reductions in plasma levels of 8-isoprostanes, VCAM-1, and peripheral mononuclear cell-derived TNF- α , below baseline normal values. There was no change in total calorie or nutrient consumption by this group, ruling out effects caused by energy changes. Importantly, the salutary changes caused by the low-AGE intervention were similar among younger and older participants. The data provide a strong impetus for subsequent studies given the potentially significant health implications of this intervention, as a simple, inexpensive, and practical method applicable to every household. Noteworthy in this investigation was the fact that the OS and AGE baseline of the healthy cohort was decreased substantially below normal by reducing the oral intake of AGEs, and that this was accomplished with no change in the calories consumed. A number of older healthy subjects

had reduced renal function (estimated glomerular filtration rate-creatinine). Importantly, these older participants did have proteinuria and had only a modest increase in OS/inflammatory markers.

CKD Subjects, Low-AGE Intervention Study

To obtain a measure of the relative impact of the intervention on subjects with different renal abilities to handle oxidants, a subgroup of subjects with established and characterized CKD unrelated to aging was included in the experimental design of the intervention.⁶⁴ The effects of the low-AGE diet in these CKD patients mimicked those in healthy participants (40%-60% reduction in inflammation) and agreed with our initial studies in subjects with diabetic and nondiabetic CKD treated with a low-AGE diet.⁶⁵

Summary of Studies in Normal Subjects and CKD Patients

These data highlighted the following: (1) there is considerable heterogeneity in the presence and degree of renal function decline in aging; (2) the measurement of glomerular filtration rate in aging needs further refinement; (3) dietary AGEs are significant contributors to the nascent AGE pool of healthy persons; (4) the chronic pressure from exogenous pro-oxidant substances gradually erodes native defenses, setting the stage for abnormally high OS and inflammation; (5) this erosion may be operative not only in patients with established increased OS, but, more importantly, in healthy subjects before the onset of end-organ decline; (6) because a physiologic intervention (dietary AGE change) effectively ameliorated increased OS, it is reasonable to conclude that increased OS may be reversible, whether in healthy aging or in diseases (such as CKD or metabolic syndrome); (7) the intervention is practical because both younger and older subjects were able to prepare low-AGE diets in their homes over a 4-month period; and (8) reduction in renal function in aging is one of the best predictors of mortality.

OVERALL SUMMARY

The data obtained from studies in experimental animals, normal aging subjects, and CKD patients suggest that oxidants contribute to changes in renal function. Both cross-sectional and longitudinal studies of aging persons suggest that a decline in renal function with age is not inevitable. Because reduced renal function is associated with cardiovascular disease, it seems very clear that interventions should be sought. The data in animals suggest that the observed decline in renal function with aging may be remediable by control of oxidants. Importantly, the levels of oxidants can be controlled by nonpharmacologic and pharmacologic means in both animals and human beings. (Fig. 1).

REFERENCES

1. Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis.* 2003; 41:1–12. [PubMed: 12500213]
2. Hsu CY, Ordonez JD, Chertow GM, Fan D, McCulloch CE, Go AS. The risk of acute renal failure in patients with chronic kidney disease. *Kidney Int.* 2008; 74:101–7. [PubMed: 18385668]
3. Finkel TH. Oxidants, oxidative stress and the biology of ageing. *Nature.* 2000; 408:239–47. [PubMed: 11089981]

4. Abbatecola AM, Ferrucci L, Grella R, Bandinelli S, Bonafe M, Barbieri M, et al. Diverse effect of inflammatory markers on insulin resistance and insulin-resistance syndrome in the elderly. *J Am Geriatr Soc.* 2004; 52:399–404. [PubMed: 14962155]
5. Cappola AR, Xue QL, Ferrucci L, Guralnik JM, Volpato S, Fried LP. Insulin-like growth factor I and interleukin-6 contribute synergistically to disability and mortality in older women. *J Clin Endocrinol Metab.* 2003; 88:2019–25. [PubMed: 12727948]
6. Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH Jr, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med.* 1999; 106:506–12. [PubMed: 10335721]
7. Reuben DB, Cheh AI, Harris TB, Ferrucci L, Rowe JW, Tracy RP, et al. Peripheral blood markers of inflammation predict mortality and functional decline in high-functioning community-dwelling older persons. *J Am Geriatr Soc.* 2002; 50:638–44. [PubMed: 11982663]
8. Stadtman ER. Role of oxidant species in aging. *Curr Med Chem.* 2004; 11:1105–12. [PubMed: 15134509]
9. Foley RN, Murray AM, Li S, Herzog CA, McBean AM, Eggers PW, et al. Chronic kidney disease and the risk for cardiovascular disease, renal replacement, and death in the United States Medicare population, 1998 to 1999. *J Am Soc Nephrol.* 2005; 16:489–95. [PubMed: 15590763]
10. Kilhovd BK, Juutilainen A, Lehto S, Ronnema T, Torjesen PA, Birkeland KI, et al. High serum levels of advanced glycation end products predict increased coronary heart disease mortality in nondiabetic women but not in nondiabetic men: a population-based 18-year follow-up study. *Arterioscler Thromb Vasc Biol.* 2005; 25:815–20. [PubMed: 15692098]
11. Santopinto JJ, Fox KA, Goldberg RJ, Budaj A, Pinero G, Avezum A, et al. Creatinine clearance and adverse hospital outcomes in patients with acute coronary syndromes: findings from the global registry of acute coronary events (GRACE). *Heart.* 2003; 89:1003–8. [PubMed: 12923009]
12. Koschinsky T, He CJ, Mitsuhashi T, Bucala R, Liu C, Buening C, et al. Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. *Proc Natl Acad Sci U S A.* 1997; 94:6474–9. [PubMed: 9177242]
13. Linden E, Cai W, He JC, Xue C, Li Z, Winston J, et al. Endothelial dysfunction in patients with chronic kidney disease results from advanced glycation end products (AGE)-mediated inhibition of endothelial nitric oxide synthase through RAGE activation. *Clin J Am Soc Nephrol.* 2008; 3:691–8. [PubMed: 18256374]
14. Fried LF, Lee JS, Shlipak M, Chertow GM, Green C, Ding J, et al. Chronic kidney disease and functional limitation in older people: health, aging and body composition study. *J Am Geriatr Soc.* 2006; 54:750–6. [PubMed: 16696739]
15. Baylis C, Corman B. The aging kidney: insights from experimental studies. *J Am Soc Nephrol.* 1998; 9:699–709. [PubMed: 9555673]
16. Wiggins JE, Goyal M, Sanden SK, Wharram BL, Shed-den KA, Misek DE, et al. Podocyte hypertrophy, “adaptation,” and “decompensation” associated with glomerular enlargement and glomerulosclerosis in the aging rat: prevention by calorie restriction. *J Am Soc Nephrol.* 2005; 16:2953–66. [PubMed: 16120818]
17. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes.* 1999; 48:1–9. [PubMed: 9892215]
18. Hamada Y, Araki N, Koh N, Nakamura J, Horiuchi S, Hotta N. Rapid formation of advanced glycation end products by intermediate metabolites of glycolytic pathway and polyol pathway. *Biochem Biophys Res Commun.* 1996; 228:539–43. [PubMed: 8920948]
19. Uribarri J, Cai W, Peppas M, Goodman S, Ferrucci L, Striker G, et al. Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. *J Gerontol A Biol Sci Med Sci.* 2007; 62:427–33. [PubMed: 17452738]
20. Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R, Dardaine V, et al. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc Natl Acad Sci U S A.* 2002; 99:15596–601. [PubMed: 12429856]
21. Vlassara H, Striker G. Glycotoxins in the diet promote diabetes and diabetic complications. *Curr Diab Rep.* 2007; 7:235–41. [PubMed: 17547841]

22. Baynes JW, Thorpe SR. Glycooxidation and lipoxidation in atherogenesis. *Free Radic Biol Med*. 2000; 28:1708–16. [PubMed: 10946212]
23. Bucala R, Makita Z, Koschinsky T, Cerami A, Vlassara H. Lipid advanced glycosylation: pathway for lipid oxidation in vivo. *Proc Natl Acad Sci U S A*. 1993; 90:6434–8. [PubMed: 8341651]
24. Voziyan PA, Hudson BG. Pyridoxamine as a multi-functional pharmaceutical: targeting pathogenic glycation and oxidative damage. *Cell Mol Life Sci*. 2005; 62:1671–81. [PubMed: 15905958]
25. Thornalley PJ. Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification—a role in pathogenesis and antiproliferative chemotherapy. *Gen Pharmacol*. 1996; 27:565–73. [PubMed: 8853285]
26. Vlassara H. Advanced glycation in health and disease: role of the modern environment. *Ann N Y Acad Sci*. 2005; 1043:452–60. [PubMed: 16037266]
27. Genuth S, Sun W, Cleary P, Sell DR, Dahms W, Malone J, et al. Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes*. 2005; 54:3103–11. [PubMed: 16249432]
28. Cai W, He JC, Zhu L, Chen X, Wallenstein S, Striker GE, et al. Reduced oxidant stress and extended lifespan in mice exposed to a low glycotxin diet: association with increased AGER1 expression. *Am J Pathol*. 2007; 170:1893–902. [PubMed: 17525257]
29. Oberg BP, McMenamin E, Lucas FL, McMonagle E, Morrow J, Ikizler TA, et al. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int*. 2004; 65:1009–16. [PubMed: 14871421]
30. Li YM, Steffes M, Donnelly T, Liu C, Fuh H, Basgen J, et al. Prevention of cardiovascular and renal pathology of aging by the advanced glycation inhibitor aminoguanidine. *Proc Natl Acad Sci U S A*. 1996; 93:3902–7. [PubMed: 8632987]
31. Wollfenbuttel BH, Boulanger CM, Crijns FR, Huijberts MS, Poitevin P, Swennen GN, et al. Breakers of advanced glycation end products restore large artery properties in experimental diabetes. *Proc Natl Acad Sci U S A*. 1998; 95:4630–4. [PubMed: 9539789]
32. Lin RY, Choudhury RP, Cai W, Lu M, Fallon JT, Fisher EA, et al. Dietary glycotoxins promote diabetic atherosclerosis in apolipoprotein E-deficient mice. *Atherosclerosis*. 2003; 168:213–20. [PubMed: 12801603]
33. Himmelfarb J, McMonagle E. Manifestations of oxidant stress in uremia. *Blood Purif*. 2001; 19:200–5. [PubMed: 11150810]
34. Peppas M, Uribarri J, Cai W, Lu M, Vlassara H. Glycooxidation and inflammation in renal failure patients. *Am J Kidney Dis*. 2004; 43:690–5. [PubMed: 15042546]
35. Schmidt AM, Hasu M, Popov D, Zhang JH, Chen J, Yan SD, et al. Receptor for advanced glycation end products (AGEs) has a central role in vessel wall interactions and gene activation in response to circulating AGE proteins. *Proc Natl Acad Sci U S A*. 1994; 91:8807–11. [PubMed: 8090728]
36. Lu C, He JC, Cai W, Liu H, Zhu L, Vlassara H. Advanced glycation endproduct (AGE) receptor 1 is a negative regulator of the inflammatory response to AGE in mesangial cells. *Proc Natl Acad Sci U S A*. 2004; 101:11767–72. [PubMed: 15289604]
37. Vlassara H. The AGE-receptor in the pathogenesis of diabetic complications. *Diabetes Metab Res Rev*. 2001; 17:436–43. [PubMed: 11757079]
38. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest*. 2001; 108:949–55. [PubMed: 11581294]
39. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler Thromb*. 1994; 14:1521–8. [PubMed: 7918300]
40. Schmidt AM, Yan SD, Yan SF, Stern DM. The biology of the receptor for advanced glycation end products and its ligands. *Biochim Biophys Acta*. 2000; 1498:99–111. [PubMed: 11108954]

41. He CJ, Koschinsky T, Buening C, Vlassara H. Presence of diabetic complications in type 1 diabetic patients correlates with low expression of mononuclear cell AGE-receptor-1 and elevated serum AGE. *Mol Med*. 2001; 7:159–68. [PubMed: 11471552]
42. Zheng F, Plati AR, Potier M, Schulman Y, Berho M, Banerjee A, et al. Resistance to glomerulosclerosis in B6 mice disappears after menopause. *Am J Pathol*. 2003; 162:1339–48. [PubMed: 12651625]
43. Zheng F, Cheng QL, Plati AR, Ye SQ, Berho M, Banerjee A, et al. The glomerulosclerosis of aging in females: contribution of the proinflammatory mesangial cell phenotype to macrophage infiltration. *Am J Pathol*. 2004; 165:1339–48.
44. Zheng F, Plati AR, Cheng Q, Bertho M, Banerjee A, Potier M, et al. Glomerular aging in females is a multi-stage reversible process mediated by phenotypic changes in progenitors. *Am J Pathol*. 2005; 162:355–63.
45. Cornacchia F, Fornoni A, Plati AR, Thomas A, Wang Y, Inverardi L, et al. Glomerulosclerosis is transmitted by bone marrow-derived mesangial cell progenitors. *J Clin Invest*. 2001; 108:1649–56. [PubMed: 11733560]
46. Zheng F, Cornacchia F, Schulman I, Banerjee A, Cheng QL, Potier M, et al. Development of albumin-uria and glomerular lesions in normoglycemic B6 recipients of db/db mice bone marrow: the role of mesangial cell progenitors. *Diabetes*. 2004; 53:2420–7. [PubMed: 15331554]
47. Tack I, Elliot SJ, Potier M, Rivera A, Striker GE, Striker LJ. Autocrine activation of the IGF-I signaling pathway in mesangial cells isolated from diabetic NOD mice. *Diabetes*. 2002; 51:182–8. [PubMed: 11756339]
48. Fornoni A, Lenz O, Striker LJ, Striker GE. Glucose induces clonal selection and reversible dinucleotide repeat expansion in mesangial cells isolated from glomerulosclerosis-prone mice. *Diabetes*. 2003; 52:2594–602. [PubMed: 14514645]
49. Fornoni A, Striker LJ, Zheng F, Striker GE. Reversibility of glucose-induced changes in mesangial cell extracellular matrix depends on the genetic background. *Diabetes*. 2002; 51:499–505. [PubMed: 11812761]
50. Zheng F, Fornoni A, Elliot SJ, Guan Y, Breyer MD, Striker LJ, et al. Upregulation of type 1 collagen by TGF-beta in mesangial cells is blocked by PPAR-gamma activation. *Am J Physiol Renal Physiol*. 2002; 282:F639–48. [PubMed: 11880325]
51. Zheng F, Zeng YJ, Plati AR, Elliot SJ, Berho M, Potier M, et al. Combined AGE inhibition and ACEi decreases the progression of established diabetic nephropathy in B6 db/db mice. *Kidney Int*. 2006; 70:507–14. [PubMed: 16775596]
52. Park C, Zhang Y, Zhang X, Wu J, Chen L, Cha DR, et al. PPARalpha agonist fenofibrate improves diabetic nephropathy in db/db mice. *Kidney Int*. 2006; 69:1511–7. [PubMed: 16672921]
53. Potier M, Karl M, Zheng F, Elliot SJ, Striker GE, Striker LJ. Estrogen-related abnormalities in glomerulosclerosis-prone mice: reduced mesangial cell estrogen receptor expression and prosclerotic response to estrogens. *Am J Pathol*. 2002; 160:1877–85. [PubMed: 12000739]
54. Elliot SJ, Karl M, Berho M, Potier M, Zheng F, Leclercq B, et al. Estrogen deficiency accelerates progression of glomerulosclerosis in susceptible mice. *Am J Pathol*. 2003; 162:1441–8. [PubMed: 12707027]
55. Karl M, Berho M, Pignac-Kobinger J, Striker GE, Elliot SJ. Differential effects of continuous and intermittent 17beta-estradiol replacement and tamoxifen therapy on the prevention of glomerulosclerosis: modulation of the mesangial cell phenotype in vivo. *Am J Pathol*. 2006; 169:351–61. [PubMed: 16877338]
56. Potier M, Karl M, Elliot SJ, Striker GE, Striker LJ. Response to sex hormones differs in atherosclerosis-susceptible and -resistant mice. *Am J Physiol Endocrinol Metab*. 2003; 285:E1237–45. [PubMed: 12915398]
57. Bobadilla NA, Tack I, Tapia E, Sanchez-Lozada LG, Santamaria J, Jimenez F, et al. Pentosan polysulfate prevents glomerular hypertension and structural injury despite persisting hypertension in 5/6 nephrectomy rats. *J Am Soc Nephrol*. 2001; 12:2080–7. [PubMed: 11562406]
58. Cai W, He JC, Zhu L, Chen X, Zheng F, Striker GE, et al. Oral glycotoxins determine the effects of calorie restriction on oxidant stress, age-related diseases, and lifespan. *Am J Pathol*. 2008; 173:327–36. [PubMed: 18599606]

59. Vlassara H. Protein glycation in the kidney: role in diabetes and aging. *Kidney Int.* 1996; 49:1795–804. [PubMed: 8743500]
60. Ruggiero C, Metter EJ, Melenovsky V, Cherubini A, Najjar SS, Ble A, et al. High basal metabolic rate is a risk factor for mortality: the Baltimore Longitudinal Study of Aging. *J Gerontol A Biol Sci Med Sci.* 2008; 63:698–706. [PubMed: 18693224]
61. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *J Am Geriatr Soc.* 1985; 33:278–85. [PubMed: 3989190]
62. Dols LF, Weimar W, Ijzermans JN. Long-term consequences of kidney donation. *N Engl J Med.* 2009; 360:2371–2. [PubMed: 19484820]
63. Pizzarelli F, Lauretani F, Bandinelli S, Windham GB, Corsi AM, Giannelli SV, et al. Predictivity of survival according to different equations for estimating renal function in community-dwelling elderly subjects. *Nephrol Dial Transplant.* 2009; 24:1197–205. [PubMed: 18988669]
64. Vlassara H, Cai W, Goodman S, Pyzik R, Yong A, Zhu L, et al. Protection against loss of innate defenses in adulthood by low AGE intake; role of a new anti-inflammatory AGE-receptor-1. *Endocrinology.* 2009 In press.
65. Uribarri J, Peppas M, Cai W, Goldberg T, Lu M, He C, et al. Restriction of dietary glycotoxins reduces excessive advanced glycation end products in renal failure patients. *J Am Soc Nephrol.* 2003; 14:728–31. [PubMed: 12595509]

Figure 1.

CKD of aging, present in C57B6 fed a regular diet (Reg) (A) was prevented by a diet low in AGE content (LAGE) (B), PAS $\times 200$, Kidney tissue AGE levels (C), Albuminuria (D), TGF β 1 (E), and Collagen IV mRNA expression (F) were decreased in 24 mo mice by a diet low in AGE content. Data are shown in ratios of test mRNA/ β -actin, by RT-PCR. (n=5/group), M $\pm$ SEM, * p < 0.05 LAGE vs. Reg (ANOVA).