

# Advanced Glycation End Products and Nephrotoxicity of High-Protein Diets

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The popularity of high-protein diets has surged recently as obesity has become more and more common in the United States and other developed nations. In view of the high prevalence of type 2 diabetes and chronic kidney disease among obese people, it is important to understand potential effects of high-protein diets on the kidney. The hypothesis that high-protein diets are nephrotoxic because of their excessive dietary advanced glycation end product (AGE) content and an increased amino acid load that enhances AGE formation *in situ* was explored. This review discusses the following evidence: (1) High-protein diets are deleterious to the kidney; (2) AGE are metabolic mediators of kidney damage; (3) dietary protein-derived AGE contribute to proinflammatory and pro-oxidative processes in diabetes and kidney disease; and (4) dietary protein-derived AGE produce functional and structural abnormalities that are involved in kidney damage. Future research should consider dietary AGE as a potential therapeutic target for kidney disease in obesity, diabetes, and perhaps other causes of chronic kidney disease.

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The popularity of high-protein diets has surged recently in the United States and other developed nations where obesity has become more and more common (1,2). High-protein diets have been promoted for weight loss, reduction in glycemia, and improvement in cardiovascular risk factors. In view of the high prevalence of type 2 diabetes and chronic kidney disease (CKD) among obese people (3,4), it is important to understand potential effects of high-protein diets on the kidney. We hypothesize that high-protein diets are nephrotoxic because they increase the circulating pool of advanced glycation end products (AGE) through an excessive dietary AGE content and an increased amino acid load that enhances AGE formation *in situ*.

## High-Protein Diets Are Deleterious to the Kidney

### Experimental Models

High-protein diets long have been known to accelerate kidney disease in animal models. Disturbances in renal hemodynamics were among the earliest abnormalities observed when protein intake was increased (5). In physiologic studies that were performed with classic micropuncture techniques, rats that were fed a high-protein diet were found to have high GFR as a result of increased glomerular perfusion and pressure (6). Persistent glomerular hypertension was shown to mediate progressive renal injury in rat models of diabetes and reduced

renal mass (6,7). Low-protein diets protected the kidney in these models. Furthermore, diabetic animals were found to be especially sensitive to dietary protein. When fed a high-protein diet, glomerular hyperfiltration and hypertension, renal injury, and loss of function were greater in diabetic compared with nondiabetic rats (7). A low-protein diet remained protective even in the setting of chronic hyperglycemia. Such data suggested an interaction between the defining feature of diabetes, hyperglycemia, and dietary protein to augment renal hemodynamic disturbances and kidney damage.

### Human Studies

Detailed physiologic studies, using precise control of glycemia and feeding, were performed to determine whether the observations that were made in animal models could be translated to humans. In studies of individuals with either type 1 or type 2 diabetes, GFR and renal plasma flow were normal after an overnight fast when the plasma glucose was clamped at an ambient level of approximately 200 mg/dl (8–10). However, when an amino acid infusion that was designed to resemble a protein meal was infused, the glomerular hyperfiltration response was much greater than normal in diabetes. This aberrant response was corrected by chronic (3 wk of intensive insulin therapy) but not acute (36-h insulin infusion) euglycemia (8,9). Therefore, hyperglycemia seems to be necessary and permissive for glomerular hyperfiltration, whereas other influences, such as fluctuating increases in amino acids in response to protein feeding, may have a more proximate effect to augment GFR in diabetes.

If these disturbances are sustained, then high dietary protein may have a deleterious influence on kidney disease, particu-

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larly in diabetes. Is there clinical evidence to support such a contention? Several large epidemiologic studies have found an association of high dietary protein intake with presence of microalbuminuria, a marker of kidney damage, and worsening renal function. The Third National Health and Nutrition Examination Survey found that level of dietary protein intake was strongly correlated with presence of microalbuminuria in those with diabetes and hypertension but not in people without diabetes (11). The Nurses' Health Study showed that women with mildly decreased kidney function (estimated GFR between 55 and 80 ml/min per 1.73 m<sup>2</sup>) had a 3.5-fold increased risk for losing kidney function when protein intake was in the highest (90 g/d) versus lowest (60 g/d) quintile (12). Importantly, the increased risk of dietary protein was confined to intake of animal meats. Vegetable or dairy-based protein sources did not adversely affect kidney function. A Dutch study of 680 white individuals between the ages of 50 and 75 yr found that each 0.1-g/kg per d increment of dietary protein intake was associated with a 20% increased risk for microalbuminuria after adjustment for age, gender, and traditional cardiovascular risk factors (13).

Several small, short-term studies of high-protein diets have been performed to evaluate effects on weight loss, but the study participants were highly selected for good health, and indicators of harm to the kidney were not examined (14–17). In our view, this sort of evidence does not alleviate concerns about the potential for high-protein diets to exacerbate kidney damage, particularly in people with diabetes and/or preexisting CKD.

Benefits of limiting protein intake have been reported in prospective studies of people with CKD, including those with diabetes. Pedrini *et al.* (18) in a meta-analysis showed that dietary protein restriction prevented progression of kidney disease (loss of function or increased albuminuria/proteinuria) in both diabetic and nondiabetic kidney diseases, although in diabetes, the benefit was even more pronounced. A subsequent meta-analysis by Kasiske *et al.* (19) that focused on measures of GFR showed that dietary protein restriction was most effective among people with diabetic kidney disease. Finally, Hansen *et al.* (20) recently performed a randomized, prospective, controlled study in people with type 1 diabetes and stage 2 CKD (inferred from levels of albuminuria and GFR). They found that a modest reduction from usual protein intake (1.02 g/kg per d) to close to the recommended dietary allowance level (0.89 g/kg per d) reduced kidney end points and death by >50%. Therefore, "restriction" may actually mean avoiding excess protein intake, which occurs so commonly in the prevalent environment of overeating in the developed world.

Despite these analyses demonstrating benefits of limiting dietary protein, the Modification of Diet in Renal Disease (MDRD) study failed to achieve its primary end point of reducing GFR loss in people with CKD of various degrees and causes (21). However, the initial GFR loss was greater in the low-protein group during the first 4 mo, presumably as a result of decreased glomerular hyperfiltration. Then, after 4 mo, GFR loss actually was slower in the low-protein group. This biphasic GFR response may have precluded demonstrating a benefit on the primary end point. Furthermore, whether GFR is the most

informative measure of kidney disease in the clinical setting is open to question. Clinical event analyses provide stronger evidence regarding effects of interventions than loss of GFR. In a secondary analysis of kidney events (ESRD or death) with follow-up for 5 to 10 mo after the end of the MDRD study intervention, a benefit of low-protein intake was noted (relative risk of events 0.63; 95% confidence interval 0.38 to 1.02;  $P = 0.056$ ) (22). Because of a modest number of events ( $n = 68$ ), this analysis was underpowered, but it suggests that people with CKD benefit from reducing intake of dietary protein.

## AGE Are Metabolic Mediators of Kidney Damage

### What Are AGE?

AGE are a heterogeneous group of compounds that are produced by nonenzymatic, sequential glycation and oxidation reactions of sugars with free amino groups on proteins, peptides, or amino acids. This sequence of events is known as the Maillard, or browning, reaction first identified in 1912 (23). It now is clear, however, that AGE compounds may form through many other pathways, including oxidation of sugars, lipids, and amino acids to create reactive aldehydes that covalently bind to proteins. The normal metabolism of glucose generates glycolytic intermediates, which also contribute to the pool of reactive aldehydes. Moreover, neutrophils and monocytes, upon inflammatory stimulation, produce myeloperoxidase and activate NADPH oxidase, which form AGE by oxidizing combinations of amino acids that are found under physiologic conditions (24,25) (Figure 1).

N<sup>ε</sup>-carboxymethyllysine (CML), pentosidine, and methylglyoxal derivatives are among some of the most well-characterized compounds that commonly are used as AGE markers. AGE can be measured by a variety of techniques. Detection of AGE fluorescence capacity is simple but nonspecific. Specific AGE can be identified and quantified by ELISA, HPLC, or mass spectrometry (26).

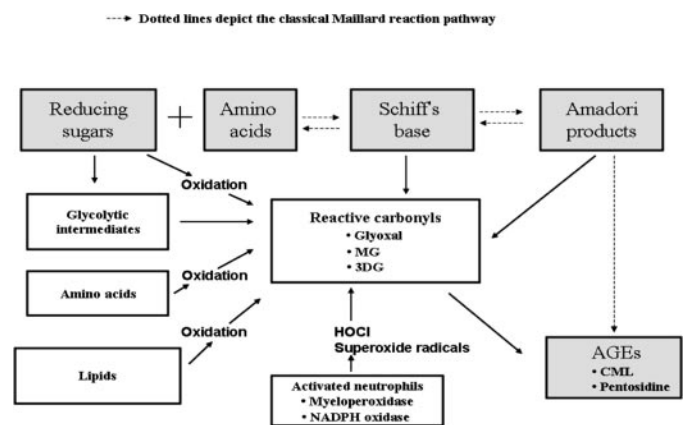


Figure 1. Biochemical reactions and common advanced glycation end product (AGE) compounds *in vivo* (23–25). MG, methyl glyoxal; DG, deoxyglucosone; HOCl, hypochlorous acid; CML, carboxymethyllysine.

### Experimental Models

Evidence for a direct role of AGE in causing kidney damage is supported by a number of experimental observations. Short-term exogenous AGE administration to normal, nondiabetic mice led to increased glomerular expression of type IV collagen and laminin, indicators of mesangial matrix expansion and basement membrane thickening (27). Furthermore, long-term treatment of normal rats with intravenous AGE-albumin induced albuminuria and morphologic changes of diabetic nephropathy, including glomerular hypertrophy, mesangial matrix expansion, and basement membrane thickening (28). When the receptor for AGE (RAGE) was overexpressed in diabetic mice, features of kidney disease (albuminuria, elevated serum creatinine, kidney hypertrophy, mesangial expansion, and glomerulosclerosis) worsened (29). Conversely, blockade of RAGE by a soluble truncated form of RAGE prevented structural and functional characteristics of nephropathy in db/db mice (30). Finally, AGE inhibitors such as aminoguanidine (28,31) and OPB-9195 (32) and the AGE breaker ALT-711 (33) prevented AGE accumulation in the kidney of diabetic rats. Albuminuria and structural changes of nephropathy also were prevented by AGE inhibitors without influencing glycemic control in these rat models of diabetes.

AGE can produce kidney damage by a variety of mechanisms that include alterations in the structure and function of proteins, as well as cellular injury. Direct cross-linking of slow-turnover proteins in extracellular matrix results in multiple abnormalities: Disrupted matrix protein structure and function (34,35); aberrant cell-matrix interactions that contribute to changes in cellular adhesion, altered cell growth, and loss of the epithelial phenotype (36); and inhibition of interactions required for self-assembly of type IV collagen and laminin (37).

AGE produce cellular injury by a cascade of receptor-dependent (e.g., RAGE) and independent events that include intracellular generation of reactive oxygen species (ROS) and a reciprocal process through which AGE and ROS mutually enhance production of one another (Figure 2) (38). ROS activate signaling pathways (e.g., mitogen-activated protein kinases, protein kinase C, Janus kinase/signal transducers and activators of transcription), which lead to proinflammatory (e.g., NF- $\kappa$ B, monocyte chemoattractant protein-1, TNF- $\alpha$ ) and profibrotic (e.g., TGF- $\beta$ , connective tissue growth factor, PDGF) effects (39–41). RAGE activation by AGE leads to transformation of tubular cells into myofibroblasts, a process that is involved in development of tubular atrophy and interstitial fibrosis (42). *In vitro* studies of cultured human podocytes also demonstrate that a glycated albumin-RAGE interaction reduces expression of nephrin, a protein that is critical for normal function of podocytes in the glomerular filtration barrier (43).

### Human Studies

AGE accumulate in the mesangial area and the glomerular capillary wall even early in diabetic nephropathy, as shown by immunohistochemical studies of kidney tissue (44). The intensity of AGE immunostaining is greatest in the areas of extensive glomerular sclerosis that is characteristic of advanced diabetic nephropathy (44). AGE also have been observed in glomeruli of

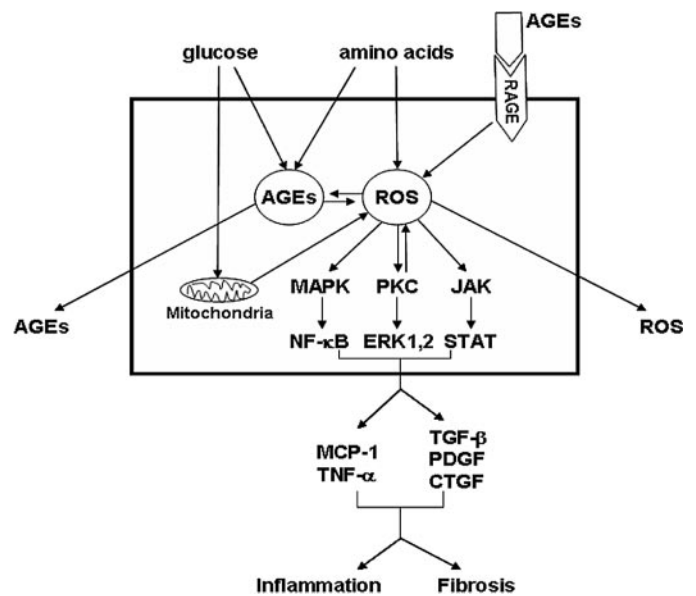


Figure 2. Cascade of events in cellular injury produced by AGE (38–42). Dietary protein is a source of preformed AGE and of amino acids that may form AGE in the circulation, kidney, and possibly other sites. RAGE, receptor for AGE; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinases; PKC, protein kinase C; JAK/STAT, Janus kinase/signal transducers and activators of transcription; ERK1,2, extracellular signal-regulated kinases 1 and 2; MCP-1, monocyte chemoattractant protein-1; CTGF, connective tissue growth factor.

nondiabetic kidney diseases (FSGS, hypertensive nephrosclerosis, and lupus nephritis) (44), but the contribution of AGE to disease progression is unexplored. This should be an important avenue of future investigation on the basis of the emerging evidence.

## Diet-Derived AGE Contribute to Proinflammatory and Pro-oxidative Processes in Diabetes and Kidney Disease

### AGE in the Diet

Recent work indicates that food, particularly protein of animal origin, is a major source of AGE (45,46). A portion of these preformed AGE is absorbed from the gastrointestinal tract as glycated amino acids and peptides. A database listing the content of CML in more than 200 food items has been compiled (45). AGE content in food is influenced by the distribution of macronutrients (proteins and fats more than carbohydrates) and by cooking conditions, including temperature, time, and moisture (45). In particular, cooking at high temperature generates a large amount of AGE because the reactions that produce these compounds are accelerated by increased heat. Culinary methods that are water based (steaming, poaching, boiling, and stewing) instead of fat based and/or browning methods (frying, broiling, and grilling) could greatly reduce AGE ingestion despite the same macronutrient content of foods. For example, a skinless chicken breast contains 692 AGE kilounits raw, 1011 AGE kilounits boiled, 5245 AGE kilounits broiled, and 6651 AGE kilounits fried (45).

Nonmeat proteins are much lower in AGE content (45). Low-AGE protein sources include reduced-fat or nonfat dairy products, soy, legumes, rice, corn, and eggs. The reason for the disparity in AGE content by source is unknown but could be due to different types of amino acids and/or fats. It is interesting that food AGE content seems to correspond with epidemiologic evidence linking consumption of animal meat but not dairy or vegetable proteins to loss of kidney function in women with early CKD (12).

### *Diabetes and Kidney Disease*

Dietary AGE are linked to multiple mechanisms of disease that are associated with diabetes and CKD (47–51). Animal studies have shown that reduced consumption of dietary AGE decreased serum AGE levels and suppressed various pathophysiologic processes, including insulin resistance in db/db (+/+) mice (47), atherosclerosis in apolipoprotein E–deficient mice (48,49), and diabetic nephropathy in nonobese diabetic and db/db (+/+) mice (50). Administration of an AGE-rich diet for 6 wk in the five-sixths nephrectomized rat increased proteinuria significantly, although it did not change GFR (51).

Common AGE compounds that are found in foods, such as CML or methylglyoxal derivatives, as their endogenous counterparts have been shown to have significant proinflammatory and pro-oxidative actions (52). LDL that was obtained from patients who had diabetes and were preexposed to a usual AGE-rich diet was shown to increase NF- $\kappa$ B activity and vascular cell adhesion molecule-1 secretion by endothelial cells, whereas LDL from patients who had diabetes and were fed an AGE-poor diet did not have such effects (53). These findings support the hypothesis that exogenous reactive AGE and AGE precursors can induce pathologic transformation of endogenous macromolecules independent of hyperglycemia. Indeed, people with diabetes or kidney disease have been shown to respond to a low-AGE diet with reduced markers of inflammation (54–56).

Although dietary AGE contribute to the internal pool, their relative importance in diabetes, kidney disease, and atherosclerotic complications remains to be elucidated. Nevertheless, the apparent deleterious nature of these compounds is a serious diet-related health concern (57). A new paradigm in which excessive AGE consumption as a result of a “Western lifestyle” produces chronic inflammation and oxidative stress has been proposed (58). Over time, excess AGE consumption likely contributes to emergence of diseases, including diabetes and CKD, that plague the developed and developing world.

## **Diet-Derived AGE Produce Functional and Structural Abnormalities that Are Involved in Kidney Damage**

### *Preformed AGE*

In addition to direct proinflammatory and pro-oxidative nephrotoxicity, AGE may alter the hemodynamics and structure of the kidney. Acute administration of an oral AGE challenge impairs systemic arterial vasodilation (flow-mediated dilation in response to brachial artery occlusion) in patients with diabetes (59) and in healthy individuals (J.U., unpublished

observations, 2005). Rats that underwent chronic intravenous administration of AGE-BSA demonstrated markedly impaired systemic vasodilatory response to acetylcholine and nitroglycerin (60). If a vasoconstrictor effect of AGE also is expressed at the level of the glomerulus, then it may not be manifest equally in the afferent and efferent arterioles. Predominant efferent vasoconstriction potentially could enhance glomerular hypertension. Such a situation occurs for the widely recognized vasoconstrictor angiotensin II (61). Chronic infusion of an AGE-modified protein in rats activated the renal renin-angiotensin system and produced glomerular hemodynamics similar to those observed in response to angiotensin II (62). If a predominantly afferent vasodilatory influence, such as increased circulating levels of amino acids after a protein meal, were to occur concomitantly, then glomerular hyperfiltration actually could be augmented.

An alternative hypothesis of glomerular hyperfiltration secondary to increased tubular size and function has been presented as an explanation for the renal hemodynamic disturbances that are characteristic of diabetes (63). This hypothesis suggests that a primary increase in proximal tubular reabsorption of sodium and/or chloride influences glomerular filtration, not *via* extracellular volume expansion but through enhanced tubuloglomerular feedback mediated by decreased distal delivery of solute to the macula densa. Habitual high intake of dietary protein or chronic infusion of an AGE-modified protein in rodents also leads to renal hypertrophy, suggesting that dietary AGE could mediate effects of high-protein diets to induce kidney growth (62,64). On the basis of this “tubular hypothesis,” increased dietary protein plausibly could predispose to glomerular hyperfiltration because of structural changes in the kidney.

Nephrotoxic effects of high-protein diets are likely to be multifactorial and overlapping. Dietary sources of AGE may be involved in many of these processes, both hemodynamic and nonhemodynamic in nature. Our hypothesis could be tested experimentally by using the rat remnant kidney model, which responds to high-protein intake with glomerular sclerosis and proteinuria (6). Assignment of rats to a high-protein diet that is either high or low in AGE content would define the respective contributions of protein *per se* versus AGE in kidney functional and structural abnormalities.

### *Formation of AGE In Situ*

Increased amounts of absorbed, nonmodified amino acids from protein-rich foods also may contribute to the circulating pool of diet-derived AGE. Amino acid oxidation or increased availability of free amino groups could induce sequential glycation and oxidative reactions, culminating in formation of AGE *in situ*. In a series of studies, we evaluated whether a key target of glomerular injury, the mesangial cell, is damaged by exposure to increased levels of amino acids that are designed to resemble a protein meal (65–67). The strength of this *in vitro* approach is that the direct cellular effects of amino acids can be separated from their renal hemodynamic effects. Exposure of mesangial cells to increased levels of amino acids causes robust proliferative and fibrotic responses, both indicators of glomer-

ular injury (65,66). These responses are remarkably similar to those that are induced by high glucose. Furthermore, the combination of increased amino acids with high glucose produces even greater increases in some glomerular injury markers.

Why should seemingly disparate metabolic disturbances, high levels of amino acids, and hyperglycemia produce similar cellular responses? We propose that generation of AGE is a potential explanation. Exposure of mesangial cells to a mixture of amino acids that resemble those that are increased in the circulation by a protein meal produces an abundance of CML, a prominent AGE (66). Inhibition of AGE formation prevents the profibrotic injury response to amino acids in mesangial cells. Classic signaling pathways (ROS, protein kinase C, and extracellular signal-regulated kinases 1 and 2) for AGE-induced cellular injury are activated by amino acids, and inhibition of these pathways prevents the injury response (Figure 2) (66). Production of AGE in response to increased levels of amino acids or glucose also is a potential explanation for activation of the renal renin-angiotensin system by high-protein diets and diabetes (62,67). For example, oxidative stress that results from sequential glycation and oxidation reactions or RAGE receptor activation could stimulate transcription of renin-angiotensin system components and angiotensin receptors (62,67–71).

Emerging evidence supports direct cellular targets of amino acid-induced kidney damage. Formation of AGE *in situ* seems to be an important mechanism for this effect. When levels of both amino acids and glucose are increased, a condition that resembles the common clinical situation of protein feeding in diabetes, the injury that is mediated by AGE may be even greater.

## Conclusion

Elucidating mechanisms of diet-induced kidney damage is critical to more effectively target therapies for lifestyle-related CKD and to improve nutritional recommendations. Compelling data from experimental models and human studies indicate that excess dietary protein promotes progressive kidney damage through increasing the AGE burden. Conversely, very low dietary protein intake may lead to malnutrition, especially in patients with advanced CKD. A prudent approach is to recommend that patients with CKD achieve the recommended dietary allowance of dietary protein (0.8 g/kg per d, or about 10% of calories) with an emphasis on protein that has high biologic value and is low in AGE. The dietary AGE load can be minimized by consuming nonmeat proteins and culinary methods that reduce AGE formation during cooking (steaming, poaching, boiling, and stewing instead of frying, broiling, or grilling) (45,54,55). Future research should address dietary AGE as a potential therapeutic target for kidney disease. Studies that span basic mechanisms to clinical treatment trials will be required to understand fully and implement best practices in this area. In the meantime, limitation of dietary AGE seems prudent in those with obesity, diabetes, and other risk factors for CKD.

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