Restriction of Dietary Glycotoxins Reduces Excessive Advanced Glycation End Products in Renal Failure Patients

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Abstract. Advanced glycation endproduct (AGE) levels are elevated in renal failure patients and may contribute to the excessive cardiovascular disease in this population. Diet-derived AGE are major contributors to the total body AGE pool. It was postulated that a reduction in dietary AGE intake might impact on the high circulating AGE levels in renal failure patients. Twenty-six nondiabetic renal failure patients on maintenance peritoneal dialysis were randomized to either a high or a low AGE diet for 4 wk. Three-day dietary records, fasting blood, 24-h urine, and dialysis fluid collections were obtained at baseline and end of study. AGE levels were determined by ELISA for N^{ϵ}-carboxymethyl-lysine (CML) and methylglyoxal-derivatives (MG). Eighteen patients completed the study. Low dietary AGE intake decreased serum CML (34%; $P < 0.002$), serum MG (35%; $P < 0.008$), CML-LDL (28%; $P < 0.011$), CML-apoB (25%; $P < 0.028$), dialysate CML (39%; $P < 0.03$), and dialysate MG output (40%; $P < 0.04$). High dietary AGE intake increased serum CML (29%; $P < 0.028$), serum MG (26%; $P < 0.09$), CML-LDL (50%; $P < 0.011$), CML-apoB (67%; $P < 0.028$), and dialysate CML output (27%; $P < 0.01$). Serum AGE correlated with BUN ($r = 0.6$, $P < 0.002$ for CML; $r = 0.4$, $P < 0.05$ for MG), serum creatinine ($r = 0.76$, $P < 0.05$ for CML; $r = 0.55$, $P < 0.004$ for MG), total protein ($r = 0.4$, $P < 0.05$ for CML; $r = 0.4$, $P < 0.05$ for MG), albumin ($r = 0.4$, $P < 0.02$ for CML; $r = 0.4$, $P < 0.05$ for MG), and phosphorus ($r = 0.5$, $P < 0.006$ for CML; $r = 0.5$, $P < 0.01$ for MG). It is concluded that dietary glycotoxins contribute significantly to the elevated AGE levels in renal failure patients. Moreover, dietary restriction of AGE is an effective and feasible method to reduce excess toxic AGE and possibly cardiovascular associated mortality.

Elevated circulating levels of advanced glycation end products (AGE) are found in renal failure patients undergoing dialysis, irrespective of the presence of diabetes (1). There is an extensive literature on the relationship between circulating AGE and atherosclerotic disease (2). Cardiovascular disease is the main cause of morbidity and mortality among dialysis patients, and only part of this increased risk can be explained by traditional risk factors (3). The suggestion has been made that uremia and/or dialysis-specific factors, including high body burden of AGE, may play a role as cardiovascular risk factors (1,2). It thus becomes clinically important to elucidate potential mechanisms for the AGE elevation to develop efficient therapies.

Increased AGE burden in renal failure patients has been attributed to either decreased renal clearance or increased endogenous formation of these compounds due to the elevated oxidant stress observed in uremia (4). Recently, it has been recognized that diet constitutes an important source of exogenous AGE (5,6). Dietary AGE content clearly depends on the nutrient composition and on the way food is processed (5,7). Modulation of oral AGE intake in human subjects or animals with or without diabetes or renal disease has been shown to modify circulating AGE levels, supporting the view that dietary AGE intake is an important contributor to the body AGE pool (6,8,9).

We postulated that the ingestion of pre-formed dietary AGE contributes significantly to the high circulating AGE levels in nondiabetic renal failure patients. To test this hypothesis, we studied a cohort of ambulatory chronic peritoneal dialysis patients without diabetes and measured circulating AGE levels before and after randomization to either a high or a low AGE content diet.

Methods

Patients and Design

This was a prospective and randomized 4-wk study. The study protocol was approval by the IRB at Mount Sinai Medical Center, New York. Twenty-six adult, stable nondiabetic peritoneal dialysis patients from this institution were recruited. None of the medications or dialysis prescriptions were modified as part of the study. Any changes that took place during the study were based on independent clinical evaluation of patients. The peritoneal dialysis fluid used was Dianeal (Baxter, Illinois) containing 40 mmol/L of lactate and a variable concentration of dextrose. Fasting venous blood, a 24-h urine, a 24-h dialysate collection, and a log containing a detailed 3-d dietary history were obtained at baseline and at the end of study.

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Patients had an initial interview with the research dietitian and were then randomized to either a high (H-AGE) or low AGE (L-AGE) diet. Subjects were individually instructed on meal planning to meet study requirements while maintaining their usual peritoneal dialysis diet instructions. To vary the AGE content, foods, particularly meat, were exposed to different cooking methods. L-AGE subjects were instructed to boil, poach, stew or steam, avoid fried entrees, and reheat food indirectly using steam in a double boiler. H-AGE participants were instructed to roast, broil and oven fry foods as usual. For both arms, written instructions and recipes were provided. The subjects were followed closely by phone calls (1 to 2 times/wk) to assure dietary compliance. Food composition from the 3-d logs was analyzed by using a nutrient software program (Food Processor version 7.1, Salem, Oregon). Dietary AGE intake was estimated by using an AGE score system, based on a database of 250 foods (9), and expressed as AGE kilounits (kU) per gram of food, whereby 1000 AGE kU were scored as 1 point AGE.

Laboratory Procedures

All routine biochemical parameters in blood, urine, and dialysate fluid were measured in the hospital clinical laboratory by standard methods. Serum, plasma, urine, and dialysate AGE were determined by ELISA using an N^{ϵ}-carboxymethyl-lysine sensitive monoclonal anti-AGE-KLH antibody (CML) and a monoclonal anti-MG-BSA antibody (MG) as described previously (9).

The 24-h urine and dialysate collections allowed the calculation of weekly urea Kt/V (an index of dialysis adequacy), normalized protein catabolic rate (nPCR; an index of protein intake), and peritoneal dialysis daily glucose absorption by standard formulas, as described before (10).

Statistical Analyses

All data are given as mean ± SEM. Differences of means between the two dietary groups were analyzed by the Mann-Whitney *U* unpaired test. Univariate correlation analyses were examined by Spearman correlation coefficient. Stepwise multiple regression analysis was performed to assess variables that were independently associated with dietary AGE intake. Statistically significant difference was defined as $P \leq 0.05$.

Results

We recruited and obtained baseline measurements in 26 patients. Eight of the 26 patients (four on L-AGE and four on H-AGE groups) did not complete the study: three developed complications unrelated to the study (persistent GI bleeding, acute myocardial infarction, and severe peritonitis). Another five patients did not respond to the dietitian phone calls to assess dietary compliance and did not show up for the follow up visit. Eighteen randomized subjects completed the study: nine subjects (six women and three men) in the H-AGE diet and nine subjects (six women and three men) in the L-AGE diet.

At baseline and at the end of study, there were no statistically significant differences between diet groups in terms of age, weight, BMI, time on peritoneal dialysis, weekly urea Kt/V, or any routine biochemical parameter.

There were no statistically significant differences between baseline and the end of study in each group or between groups in terms of peritoneal dialysate volume inflow (10 *versus* 9.8 L/d in the L-AGE group; 10.8 *versus* 10.9 L/d in the H-AGE group), peritoneal dialysate fluid glucose inflow (230 *versus* 220 g/d in the L-AGE group; 236 *versus* 243 g/d in the H-AGE group), and peritoneal glucose load received by the patient (142 *versus* 135 g/d in the L-AGE group; 122 *versus* 139 g/d in the H-AGE group).

Dietary changes between baseline and end of the experimental periods, as estimated by 3-d dietary records are shown in Table 1.

During the L-AGE diet, serum CML, serum MG, CML-LDL, CML-apoB levels, dialysate CML, and dialysate MG output diminished significantly (34%, $P < 0.021$; 35%, $P < 0.008$; 28%, $P < 0.011$; 25%, $P < 0.028$, 39%, $P < 0.03$; 40%,

Table 1. Nutrient and glycotoxin intake based on dietary records

Nutrient	Diets	Baseline	End	% change	*P*
Calories (kcal/d)	L-AGE	1487 ± 246	1431 ± 248	−4 ± 8	0.74
	H-AGE	1749 ± 248	1987 ± 243	16 ± 3[a]	0.01
Protein (g/d)	L-AGE	70 ± 12	56 ± 10	−21 ± 5[a]	0.03
	H-AGE	82 ± 12	93 ± 15	13 ± 4[a,b]	0.02
Fat (g/d)	L-AGE	53 ± 12	42 ± 9	−21 ± 6[a]	0.03
	H-AGE	58 ± 7	77 ± 12	38 ± 17[a,b]	0.03
Saturated fat (g/d)	L-AGE	18 ± 3	11 ± 2	−34 ± 6[a]	0.02
	H-AGE	21 ± 4	26 ± 4	34 ± 9[b]	0.07
Carbohydrates (g/d)	L-AGE	180 ± 33	213 ± 38	28 ± 24	0.4
	H-AGE	206 ± 29	233 ± 28	14 ± 5[a]	0.01
AGE (points/d)[c]	L-AGE	12.4 ± 1.5	5.5 ± 0.9	−55 ± 6[a]	0.01
	H-AGE	13.8 ± 3	17 ± 3	40 ± 21[b]	0.15

[a] Statistically different percent of change between baseline and end of study within each group (*P* values reflect this change).
[b] Statistically significant difference between the study groups at end of study (*P* values not shown, but always <0.05).
[c] Food AGE content was based on a scoring system using points/day, whereby 1 point = 1000 AGE kU (see Materials and Methods section).

$P < 0.04$, respectively). During the H-AGE diet serum CML increased by 29% ($P < 0.028$), serum MG by 26% ($P < 0.09$), CML-LDL by 50% ($P < 0.011$), CML-apoB by 67% ($P < 0.028$), and dialysate CML by 27% ($P < 0.01$) (Figure 1). None of these parameters was significantly different between both groups at baseline, but all of them became significantly different at the end of the study. The daily dialysate and urinary output of CML and MG-derivatives decreased during the L-AGE diet and increased during the H-AGE diet, although only the dialysate differences for CML in both groups and MG in the L-AGE group reached statistical significance.

Regression analysis revealed a highly significant correlation between serum CML and serum MG-derivatives and BUN ($r = 0.6$, $P < 0.002$ for CML; $r = 0.4$, $P < 0.05$ for MG), serum creatinine ($r = 0.76$, $P < 0.05$ for CML; $r = 0.55$, $P < 0.004$ for MG), total protein ($r = 0.4$, $P < 0.05$ for CML; $r = 0.4$, $P < 0.05$ for MG), albumin ($r = 0.4$, $P < 0.02$ for CML; $r = 0.4$, $P < 0.05$ for MG), and phosphorus ($r = 0.5$, $P < 0.006$ for CML; $r = 0.5$, $P < 0.01$ for MG). Dietary AGE score correlated with dietary protein ($r = 0.6$, $P < 0.007$), fat ($r = 0.57$, $P < 0.01$), saturated fat ($r = 0.6$, $P < 0.0040$), and caloric intake ($r = 0.58$, $P < 0.01$). Multiple regression analysis revealed that dietary content of saturated fat was the variable that best predicted dietary AGE score ($r^2 = 0.75$; $P = 0.000$).

Discussion

The current study indicates that even short-term modifications of dietary AGE intake can significantly alter circulating AGE levels in renal failure patients on maintenance peritoneal dialysis. These findings support the hypothesis that exogenous glycotoxins derived from common diets, including those recommended for dialysis patients, play a significant role in maintaining high circulating AGE levels in uremia.

There is extensive experimental evidence from animal studies showing significant changes in circulating AGE levels as a result of dietary AGE modulations (6,8,9). Recently, it has been shown that circulating AGE levels decreased after dietary AGE restriction in diabetic patients with normal renal function (9). In the present study, we found a comparable effect of the low AGE intake in nondiabetic subjects with renal failure. In addition, the current group consumed self-selected diets prepared by them at home indicating that these dietary modifications are feasible as an intervention in this population.

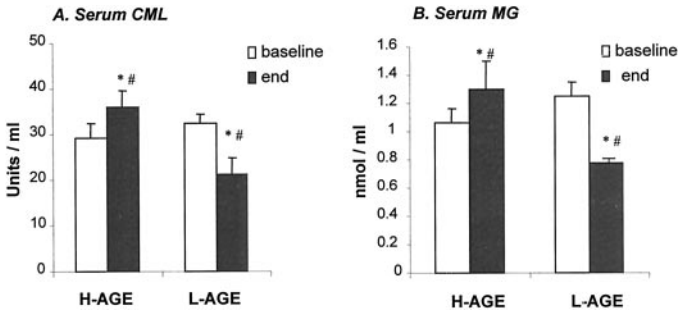

Figure 1. Serum advanced glycation endproduct (AGE) levels in peritoneal dialysis patients exposed to high- and low-AGE diet. At baseline and after 4-wk exposure to either high (H-AGE) or low-AGE (L-AGE) diet, fasting serum samples were tested for AGE using ELISA for N^{ϵ}-carboxymethyl-lysine (CML; panel A) and MG-derivatives (MG; panel B). *Statistically significant difference between baseline and end of study in each group ($P \leq 0.05$). # Statistically significant difference at the end of study between the two groups ($P \leq 0.05$). Data are shown as means ± SEM.

The positive correlation between circulating AGE and parameters directly depending on food intake, such as BUN, serum phosphorus, and potassium, further supports the postulation that diet represents an important source for these compounds. The correlation of circulating AGE with nutritional indicators such as serum total protein, albumin, and creatinine supports the notion that AGE may prove useful as markers of nutritional status.

The simultaneous and parallel changes in circulating, urinary, and dialysate AGE levels were most likely attributable to diet, as other potentially confounding factors were eliminated. Diabetic patients were excluded, the daily amount of glucose absorbed during peritoneal dialysis did not differ significantly between baseline and experimental periods, and blood glucose and lipid levels did not change during the study period. Moreover, there was no obvious evidence for any independent changes in the oxidative state to influence AGE levels in these patients. Although not tested, a reduction in serum inflammatory markers was found in response to the same low AGE diet in diabetic patients (9), suggesting that a similar effect may have occurred in the current patients.

The feasibility of an intervention that lowers circulating AGE levels in renal failure patients should be appealing to clinicians in view of the extremely high mortality from cardiovascular disease in this population (3), the experimentally proven toxicity of AGE (1), and the ineffectiveness of available therapy. Although a large body of data suggests strongly that circulating AGE may be an important cardiovascular risk factor in renal failure (1,2), a recent clinical study (11) showed a negative correlation between serum CML levels and cardiovascular mortality in dialysis patients indicating the need for further studies.

AGE can also be generated in the peritoneal cavity during peritoneal dialysis (12). The fact that changes in circulating and dialysate AGE levels followed in parallel the changes in dietary AGE, while peritoneal dialysis prescription remained unchanged, suggests that the magnitude of intraperitoneal production of AGE is relatively modest.

Changes in peritoneal membrane function associated with changes in serum and dialysate AGE have been described (13), but the short duration of our study made any such changes unlikely.

In conclusion, dietary restriction of AGE may prove a reasonable method to reduce the excessive burden of toxic AGE in vital tissues and presumably the morbidity and mortality associated with their accumulation. In addition, this short-term study shows that this intervention is feasible in renal failure patients. Although the current intervention dealt exclusively with chronic peritoneal dialysis patients, these findings should

be applicable to patients on hemodialysis or to chronic renal failure patients in general.

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