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Authors
Shinaberger, Christian S
Kilpatrick, Ryan D
Regidor, Deborah L
et al.

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Longitudinal Associations Between Dietary Protein Intake and Survival in Hemodialysis Patients

Christian S. Shinaberger, MPH, Ryan D. Kilpatrick, MS, Deborah L. Regidor, MPH, Charles J. McAllister, MD, Sander Greenland, DrPH, Joel D. Kopple, MD, and Kamyar Kalantar-Zadeh, MD, MPH, PhD

● ***Background:*** **Decreased dietary protein intake may be associated with increased mortality risk in individuals with kidney failure undergoing maintenance hemodialysis (MHD). We hypothesized that longitudinal changes in dietary protein intake have independent associations with survival in MHD patients.** ***Methods:*** **The relation between urea kinetic–based normalized protein nitrogen appearance (nPNA) and all-cause and cardiovascular mortality was examined in a 2-year (July 2001 to June 2003) cohort of 53,933 MHD patients from virtually all DaVita dialysis clinics in the United States, using both conventional and time-dependent (repeated-measure) Cox models to estimate death hazard ratios for quarterly averaged nPNA categories controlled for case-mix, comorbidity, dialysis dose (Kt/V), and available markers of malnutrition-inflammation complex syndrome (MICS).** ***Results:*** **The best survival was associated with nPNA between 1.0 and 1.4 g/kg/d, whereas nPNA less than 0.8 or greater than 1.4 g/kg/d was associated with greater mortality in almost all models. Adjustment for MICS mitigated the associations substantially. A decrease in protein intake during the first 6 months in patients with an nPNA in the 0.8- to 1.2-g/kg/d range was associated incrementally with greater death risks in the subsequent 18 months, whereas an increase in nPNA tended to correlate with reduced death risk.** ***Conclusion:*** **Low daily protein intake or decrease in its magnitude over time is associated with increased risk for death in MHD patients. Whether the association between time-varying protein intake and survival is causal or a consequence of anorexia secondary to MICS or other factors needs to be explored further in interventional trials.** ***Am J Kidney Dis*** **48:37-49.**

INDEX WORDS: Hemodialysis (HD); protein nitrogen appearance; cardiovascular death; time-dependent Cox model; malnutrition-inflammation complex syndrome.

IN INDIVIDUALS WITH such chronic disease states as chronic renal failure, decreased dietary protein intake may be associated with poor survival.[1] Low protein intake may be caused by anorexia as a result of inflammation and may lead to protein-energy malnutrition.[2] The protein-energy malnutrition per se may engender or aggravate inflammation.[3] The so-called malnutrition-inflammation complex (or cachexia) syndrome (MICS) has been implicated as a powerful indicator of poor clinical outcome in maintenance hemodialysis (MHD) patients.[4] Among components of MICS, manifestations of protein-energy malnutrition have been studied extensively, especially because they are common and correlate strongly with poor outcome in MHD patients.[5,6] This association may be a cause of the paradoxical relations between traditional cardiovascular (CV) risk factors and death risk in MHD patients[7]; MHD patients with greater protein and, possibly, energy intake usually have a greater body mass index (BMI), greater serum

From the Division of Nephrology and Hypertension, Los Angeles Biomedical Institute at Harbor-UCLA Medical Center, Torrance; School of Public Health, University of California; David Geffen School of Medicine at UCLA, Los Angeles; and DaVita Inc, El Segundo, CA.

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Address reprint requests to Kamyar Kalantar-Zadeh, MD, PhD, MPH, Division of Nephrology and Hypertension, Harbor-UCLA Medical Center, 1000 W Carson St, Torrance, CA 90509-2910. E-mail: kamkal@ucla.edu

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cholesterol levels, probably greater protein mass and intake, and also better survival.[4,7]

By virtue of end-stage renal failure, MHD patients cannot excrete significant amounts of urinary nitrogen. Hence, the rate of increase in serum urea nitrogen levels between 2 subsequent hemodialysis sessions is a reliable function of dietary nitrogen intake, provided there is no negative or positive nitrogen balance. This indirect, but conveniently available, measure of protein intake is referred to as the urea kinetic–based protein equivalent of total nitrogen appearance (PNA) or protein catabolic rate (PCR), which usually is normalized for the ideal body weight: hence, nPNA or nPCR. Although nPNA as a measure of dietary protein intake may have a bearing on clinical outcome in MHD patients, many studies that examined the association between nPNA and survival evaluated only the effect of the initial or baseline nPNA, obtained at the start of the study, on subsequent mortality. We are unaware of other studies that examined the effect of changes in nPNA over time on mortality with or without multivariate adjustment for other elements of MICS, which also may vary over time.

Using time-dependent multivariate models, we examined whether nPNA changes over time are predictors of survival in MHD patients independent of baseline nPNA and changes in other covariates. We hypothesized that survival would improve linearly across the higher nPNA groups independent of case-mix characteristics, comorbidity, dialysis dose, and other confounders. We also hypothesized that an increase in nPNA over time would be associated with better survival, whereas a decrease in nPNA over time would be a predictor of increased death in MHD patients.

METHODS

Patients

We examined the national database of DaVita Inc, the second largest dialysis care provider in the United States. Database creation was described elsewhere.[8] In summary, this database includes information on approximately 40,000 maintenance dialysis patients at any given time. A 2-year cohort was created by using data collected between July 1, 2001, and June 30, 2003. All repeated measures for each patient within a given calendar quarter (13-week interval) were averaged to obtain 1 quarterly mean value per patient per variable. The study was approved by the Institutional Review Committees of Harbor-UCLA and DaVita Inc.

Patient postdialysis weight from each hemodialysis treatment was averaged during each 13-week quarter, and BMI (weight in kilograms divided by height squared in square meters) was calculated. Age was estimated by using date of birth and the first day of the entry quarter. Five race/ethnic groups were defined: Caucasians (including non-Hispanic whites and Middle Easterners); self-described blacks (including African Americans and sub-Saharan Africans); Asians (including Pacific Islanders); American Indians; and others.

History of tobacco smoking and preexisting CV and non-CV comorbid conditions were obtained by linking the DaVita database to Medical Evidence Form 2728 of the United States Renal Data System[9] and categorized into 10 comorbid conditions: (1) ischemic heart disease, (2) congestive heart failure, (3) status post cardiac arrest, (4) status post myocardial infarction, (5) pericarditis, (6) cardiac arrhythmia, (7) cerebrovascular events, (8) peripheral vascular disease, (9) chronic obstructive pulmonary disease, and (10) cancer.

Cohort time included number of days that a patient participated in the cohort and was a number between 1 and 731 days. Dialysis vintage is defined as duration of time between the first day of dialysis treatment and the first day that the patient entered the cohort. Four categories of vintage were formed: (1) first 6 months, (2) between 6 and 24 months, (3) between 2 and 5 years, and (4) longer than 5 years. Entry quarter is defined as the first quarter in which a patient's dialysis vintage was greater than 3 months for at least half the duration of the quarter, as in our previous studies.[8,10-12] By implementing this criterion, a patient who did not remain in the cohort beyond the first 3 months of MHD was excluded. Computerized causes of death, reflecting the reported information in the cause of death form (Form 2746), were obtained from the United States Renal Data System and summarized into 5 main categories: CV, infectious, gastrointestinal, cancer related, and others/unspecified/unknown. CV death included death from myocardial infarction, cardiac arrest, heart failure, cerebrovascular accident, and other cardiac events.

Laboratory Methods

All laboratory measurements were performed by DaVita Laboratories in Deland, FL, by using standardized and automated methods. For each laboratory measure, the average of all available values obtained within any given calendar quarter was used in all analyses. nPNA and Kt/V (single pool) were calculated by using urea kinetic modeling (UKM) formulas. Commonly used alternate formulas, representing simplified UKM equations, are as follows[13,14]:

$$Kt/V = -\ln(R - 0.008 * t) + [(4 - 3.5 * R) * UF/W)]$$

$$PNA = C_0/[25.8 + (1.15/[Kt/V]) + 56.4/(Kt/V)] + 0.168$$

where R is the ratio of postdialysis to predialysis serum urea nitrogen, t is time of dialysis in hours, UF is the amount of ultrafiltration (in liters), W is postdialysis weight (in kilograms), and C_0 is predialysis concentration of serum urea nitrogen. The nPNA unit is gram of net protein degradation per kilogram of body weight per day. However, the UKM formulas used in DaVita laboratories to calculate Kt/V and

nPNA are more complex, and computational software programs routinely are used. In general, PNA is a function of urea generation rate (G) and the calculated total body water (TBW), both of which are estimated during the UKM computations[15]:

$$PNA = (5.423 * G)/(0.001 * TBW) + 0.168$$

All calculated PNA values are normalized (nPNA) to an idealized body weight based on the postdialysis dry weight of the patient and other anthropometric and demographic variables entered into the UKM software program.

Most blood samples were collected predialysis, with the exception of the postdialysis serum urea nitrogen level to calculate urea kinetics. Blood samples were drawn by using uniform techniques in all DaVita dialysis clinics across the nation and were transported to the DaVita Laboratory in Deland, FL, usually within 24 hours. Most laboratory values, including complete blood cell counts and serum levels of urea nitrogen, albumin, creatinine, ferritin, and total iron-binding capacity (TIBC), were measured monthly. Both nPNA and Kt/V were estimated monthly. Serum ferritin was measured quarterly. Hemoglobin was measured weekly to biweekly in most patients. Ten categories of nPNA were created: less than 0.6 g/kg/d, 1.4 g/dL or greater, and 8 incremental categories of 0.1 g/dL in between. Values less than the 0.25th or greater than the 99.75th percentile levels of nPNA were excluded because of increased likelihood of errors of such outliers. Patients with missing nPNA values in all 8 quarters were excluded.

The following 10 time-varying (quarterly changing) laboratory variables with up to 8 repeated measures per patient during the 2-year cohort time also were included in the models as potential confounders: (1) serum ferritin, (2) serum albumin, (3) serum TIBC, (4) serum creatinine, (5) serum phosphorus, (6) serum calcium, (7) serum bicarbonate, (8) blood hemoglobin, (9) peripheral white blood cell count (WBC), and (10) lymphocyte percentage. These measures are related to MICS and were associated in other studies with important outcomes in dialysis patients, as described elsewhere.[8,10,11,16,17]

Statistical and Epidemiological Methods

Both fixed-covariate (time-independent) Cox models using baseline data and time-dependent Cox models using repeated (quarterly varying) measures were examined,[18] and results were compared. Eight quarterly data sets were merged by using unique patient identifiers. A nonconcurrent cohort with quarterly units and quarterly values for each time-varying variable was formed to include all existing MHD patients of the first quarter (q1) and all new MHD patients of the subsequent quarters (q2 through q8). In addition to 8 quarterly values for every variable, a baseline value was created for each measure by left-truncating the first available value of the entry quarter for each patient. For time-dependent models, a dynamic (open) cohort that incorporated influx (new patients) and outflux (died or left censored) patients was studied. The reference nPNA category for all analyses was 0.9 or greater and less than 1.0 g/kg/d. This category was chosen because it was the modal category, it had the highest number of death cases, and it produced the most precise comparison with higher and lower nPNA categories.

For each analysis, 3 types of models were examined based on the level of multivariate adjustment: (1) unadjusted models included only nPNA categories, entry quarter, and mortality data; (2) case-mix–, comorbidity-, and dialysis dose–adjusted models also included age; sex; race and ethnicity; diabetes mellitus; vintage categories; primary insurance (Medicare, Medicaid, private, and others); marriage status (married, single, divorced, widowed, and other); standardized mortality ratio of the dialysis clinic during entry quarter; Kt/V (single pool); residual renal function during the entry quarter, ie, urinary urea clearance; dialysis access (catheter versus arteriovenous shunt); history of smoking; and 10 preexisting comorbid conditions (described previously); and (3) case-mix– and MICS-adjusted models included all mentioned covariates, as well as 12 indicators of nutritional status and inflammation, including BMI, administered dose of erythropoietin (EPO) as a surrogate of inflammation,[19] and 10 previously mentioned laboratory values. In time-dependent models, in addition to time-varying quarterly nPNA categories, 12 indicators of MICS and Kt/V were entered as quarterly time-varying variables, with up to 8 values per calendar quarter per patient. Missing covariate data (<5% for all variables except serum ferritin level, smoking, and 10 comorbid states) were input by the mean or median of the existing values, whichever was most appropriate. For variables with missing values greater than 5%, such as comorbid states, a dummy variable also was created to indicate the missing status for each variable.

Standard descriptive statistics also were performed, and multiple linear regression models were fitted to construct partial correlations. Because of the very large numbers involved, most associations and interactions that we examined have very low *P* values. All descriptive and multivariate statistics were carried out using SAS, version 8.02 (SAS Institute Inc, Cary, NC).

RESULTS

A total of 69,819 MHD patients were in the database during the 2-year study. After excluding patients who did not remain beyond 3 months of MHD, ie, 5,600 patients from the first 7 calendar quarters and 5,870 patients from the last quarter (q8), 58,349 MHD patients remained, of whom 53,933 MHD patients had the required data for the planned analyses; 35,050 patients (65%) originated from the q1 data set and the rest from the subsequent quarters (q2 through q8). Table 1 lists baseline characteristics of patients divided into 2 categories of nPNA: less than 1.0 g/kg/d (n = 28,540; 53%) and 1 g/kg/d or greater (n = 25,393; 47%). The latter nPNA range is the recommended target by the National Kidney Foundation–Kidney Disease Outcomes Quality Initiative guidelines.[1] MHD patients with low protein intake were older and included more blacks, but

Table 1. Baseline Data for 53,933 MHD Patients at Baseline of the 2-Year Cohort

Variable	nPNA < 1 g/kg/d (n = 28,540)	nPNA ≥ 1 g/kg/d (n = 25,393)
Age (y)	61.8 ± 15.6	59.9 ± 15.2
>65 y (%)	47	41
Sex (% women)	47	45
Diabetes mellitus (%)	44	47
Race and ethnicity:		
Caucasians (%)	38	36
Blacks (%)	36	28
Asians (%)	3	6
Hispanics (%)	15	21
Vintage (time on dialysis):		
<6 mo (%)	43	39
6-24 mo (%)	21	23
2-5 y (%)	23	25
>5 y (%)*	13	13
Patients with a catheter (*v* arteriovenous shunt)	23	22
Causes of death:		
Cardiovascular (%)	50	54
Infectious (%)†	14	12
Posthemodialysis weight (kg)	74.1 ± 20.0	73.5 ± 19.5
BMI (kg/m^2)	25.1 ± 3.7	25.0 ± 3.7
Kt/V (single pool)	1.5 ± 0.3	1.6 ± 0.3
Serum albumin (g/dL)	3.68 ± 0.44	3.84 ± 0.34
Creatinine (mg/dL)	8.3 ± 3.2	9.8 ± 3.3
Ferritin (ng/mL)	492 ± 303	527 ± 306
Phosphorus	5.5 ± 1.4	6.0 ± 1.6
Calcium†	9.2 ± 0.7	9.2 ± 0.7
Bicarbonate	22.3 ± 2.7	21.2 ± 2.7
TIBC (mg/dL)	196 ± 43	207 ± 39
Blood hemoglobin (g/dL)	11.9 ± 1.3	12.1 ± 1.2
WBCs (per fl)	7.3 ± 2.4	7.3 ± 2.3
Lymphocyte (% of total WBC)†	21.0 ± 7.7	21.0 ± 7.5
EPO dose (U/wk)	16,562 ± 9,863	14,298 ± 8,166

NOTE. *P* for the difference between the 2 groups are < 0.001 unless specified. To convert serum creatinine in mg/dL to μmol/L, multiply by 88.4; albumin and hemoglobin in g/dL to g/L, multiply by 10; ferritin in ng/mL to μg/L, multiply by 1.

*$P > 0.05$.

†$0.001 < P < 0.05$.

fewer Hispanics. Dialysis dose and values for all laboratory markers except serum bicarbonate were lower in patients with nPNA less than 1 g/kg/d, who received greater EPO doses.

Table 2 lists bivariate (unadjusted) and multivariate-adjusted correlation coefficients between nPNA and some clinically relevant variables in the baseline quarter of the cohort. Kt/V and serum creatinine, bicarbonate, and phosphorus concentrations showed the strongest correlations with nPNA. Table 3 lists the 10 nPNA categories among the 53,933 MHD patients. Both all-cause and CV mortality showed decreasing rates across increasing nPNA categories, whereas serum albumin level was progressively greater.

Table 4 lists hazard ratios for death for time-varying nPNA categories. In almost all models, nPNA less than 0.9 or greater than 1.4 g/kg/d was associated with greater all-cause and CV mortality compared with the reference group. The nPNA value between 1.1 and 1.4 g/kg/d tended to be associated with the lowest all-cause mortality in the unadjusted and case-mix–adjusted models. When additional adjustment for MICS markers took place, no obvious multivariate-adjusted survival advantage among subgroups of the entire spectrum of 0.9- through 1.39-g/kg/d range was notable. Figure 1 shows death hazard ratios of nPNA increments by using time-independent models in all 53,933 MHD patients across the nation. To remove the possible confounding effect of residual renal function, all analyses were performed in 47,119 MHD patients without no-

Table 2. Bivariate (Unadjusted) and Multivariate Correlation Coefficients Between nPNA (nPCR) and Some Relevant Variables at Baseline Calendar Quarter in 53,933 MHD Patients

Variable	Pearson Correlation *r*	Multivariate Adjusted Correlation*
Age	−0.07	−0.08
Kt/V (dialysis dose)	**+0.28**	**+0.29**
BMI	+0.01†	+0.01†
Serum albumin	**+0.26**	+0.09
Phosphorus	**+0.24**	**+0.17**
Calcium	+0.01	−0.02
Bicarbonate	**−0.23**	**−0.16**
TIBC	**+0.17**	**+0.16**
Creatinine	**+0.26**	**+0.37**
Ferritin	+0.03	+0.04
Blood hematocrit	+0.06	+0.01‡
WBCs	+0.02	+0.01†
Lymphocyte %	−0.01‡	−0.01†
Administered EPO	−0.09	+0.02

NOTE. $r > 0.15$ are in bold type. All $P < 0.001$ unless noted otherwise.

*The multiple regression model includes all case-mix and MICS covariates (see text).

†*P* between 0.001 and 0.05.

‡$P > 0.05$.

Table 3. Categories of 3-Month Averaged nPNA (nPCR), Death Rates, and Serum Albumin Levels at Baseline (First Prevalent Quarter) in 53,933 MHD Patients

nPNA (nPCR) Range (g/kg/d)	Group Size (% total)*	All-Cause Death (% in 2 y)*	CV Death (% in 2 y)*	Serum Albumin (g/dL)†
<0.60	2,086 (4)	790 [38]	293 [14]	3.32 ± 0.58
≥0.60 and <0.70	3,524 (7)	1,123 [32]	434 [12]	3.55 ± 0.48
≥0.70 and <0.80	6,026 (11)	1,785 [30]	753 [13]	3.66 ± 0.42
≥0.80 and <0.90	8,047 (15)	2,118 [26]	894 [11]	3.74 ± 0.39
≥0.90 and <1.0‡	8,857 (16)	2,130 [24]	929 [11]	3.78 ± 0.37
≥1.0 and <1.1	8,240 (15)	1,856 [23]	831 [10]	3.86 ± 0.35
≥1.1 and <1.2	6,338 (12)	1,363 [22]	603 [10]	3.84 ± 0.34
≥1.2 and <1.3	4,591 (9)	958 [21]	452 [10]	3.86 ± 0.33
≥1.3 and <1.4	2,711 (5)	560 [21]	251 [9]	3.86 ± 0.33
≥1.4	3,513 (6)	749 [21]	365 [11]	3.87 ± 0.33

*Values in parentheses present the proportion of MHD patients in each nPNA category. Values in brackets indicate the crude death rate in the indicated group during the 2 years of observation.

†Serum albumin and nPNA expressed as mean ± SD.

‡Reference group.

table residual renal function, ie, urinary urea clearance = 0 (Fig 2). Analyses were repeated for incident (vintage < 6 months) and prevalent (vintage > 6 months) patients separately, as shown in Figs 3 and 4, respectively. All models also were examined for CV death, which resulted in similar associations (data not shown). We also performed separate models for continuous (noncategorized) nPNA values, which resulted in a death hazard ratio of 0.94 (95% confidence interval, 0.93 to 0.95; $P < 0.0001$) for each 0.1-g/kg/d increase in nPNA for the case-mix–adjusted model.

Of 35,050 MHD patients who entered the cohort in q1 and formed the longest subcohort, 31,587 patients survived and remained in the cohort through the first several months. This allowed estimation of the rate of change in nPNA values during the first 6-month interval. Table 5 lists rates of nPNA change in 5 distinct categories. The stable category included 14,280 MHD patients (45%) for whom nPNA had only a

Table 4. Time-Dependent Association Between nPNA (nPCR) in Each Quarter and Death During the 2-Year Cohort of 53,933 MHD Patients

All-Cause Mortality	Unadjusted		Case-Mix Adjusted		Case-Mix and MICS Adjusted	
nPCR (g/kg/d)	Hazard Ratio (95% Confidence Interval)	*P*	Hazard Ratio (95% Confidence Interval)	*P*	Hazard Ratio (95% Confidence Interval)	*P*
<0.6	3.12 (2.94-3.42)	<0.0001	2.14 (1.98-2.32)	<0.0001	1.34 (1.23-1.46)	<0.0001
≥0.6 and < 0.7	2.08 (1.93-2.24)	<0.0001	1.56 (1.45-1.68)	<0.0001	1.15 (1.07-1.24)	0.0004
≥0.7 and < 0.8	1.58 (1.47-1.68)	<0.0001	1.33 (1.25-1.43)	<0.0001	1.14 (1.07-1.22)	0.0002
≥0.8 and < 0.9	1.24 (1.16-1.33)	<0.0001	1.15 (1.08-1.23)	<0.0001	1.07 (1.00-1.14)	0.06
≥0.9 and <1.0*	1.00	NA	1.00	NA	1.00	NA
≥1.0 and <1.1	0.91 (0.85-0.98)	0.01	0.98 (0.92-1.05)	0.57	1.04 (0.97-1.11)	0.27
≥1.1 and <1.2	0.85 (0.79-0.91)	<0.0001	0.93 (0.86-1.00)	0.06	0.99 (0.92-1.07)	0.86
≥1.2 and <1.3	0.76 (0.69-0.82)	<0.0001	0.89 (0.82-0.98)	0.01	1.03 (0.95-1.13)	0.45
≥1.3 and <1.4	0.73 (0.66-0.81)	<0.0001	0.90 (0.81-1.00)	0.05	1.01 (0.91-1.12)	0.89
≥1.4	1.03 (0.94-1.12)	0.54	1.29 (1.18-1.40)	<0.0001	1.34 (1.23-1.47)	<0.0001

NOTE. Covariates in case-mix include age, sex, race/ethnicity, diabetes mellitus, vintage, insurance, marital status, standardized mortality ratio, Kt/V, tobacco use, residual urine, and history of 10 comorbid conditions. Covariates in MICS include serum albumin, TIBC, ferritin, creatinine, calcium, phosphorus, bicarbonate, blood hemoglobin, WBCs, lymphocyte %, BMI, and administered erythropoietin dose.

Abbreviation: NA, not applicable.

*Reference group.

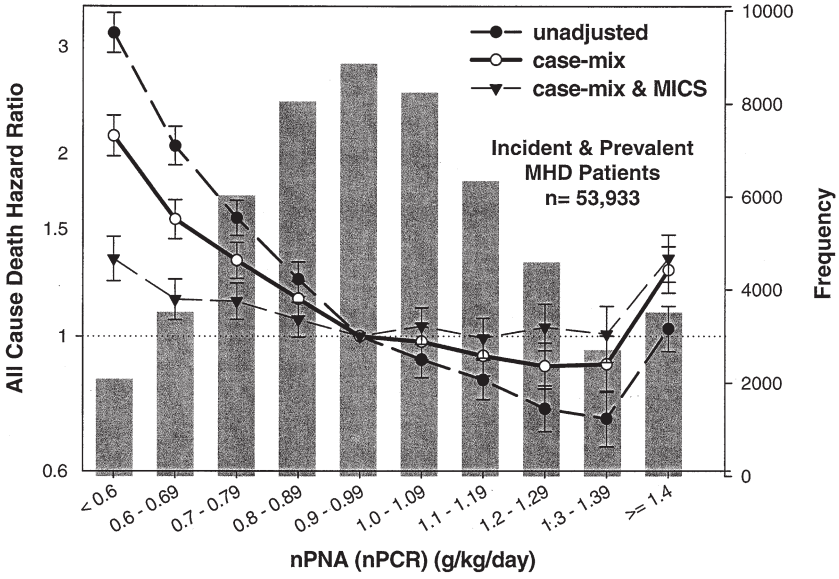

Fig 1. Association between nPNA and 2-year mortality in all 53,933 MHD patients of DaVita dialysis clinics based on time-dependent models using quarterly varying repeated measures.

minimal change, ie, within the range of +0.1 to −0.1 g/kg/d during the 6-month interval. Patients for whom nPNA decreased or increased by at least 0.1 g/kg/d or more were subdivided further into 2 categories.

As listed in Table 5, patients for whom nPNA increased by 0.1 g/kg/d or greater during 6 months had the lowest baseline nPNA (0.95 g/kg/d) at their entry, whereas patients for whom nPNA decreased had the highest baseline value. To circumvent this "regression-to-the-mean" phenomenon (which may confound survival associations), Cox regression modeling for changes in the first 6 months was restricted to patients with baseline nPNA between 0.8 and 1.2 g/kg/d (n = 20,854), approximately corresponding to 1 SD variation around the nPNA mean. Table 6 and Fig 5 show hazard ratios for death in the subsequent 18 months for changes in nPNA during the first 6 months of the cohort. Death hazard ratios across decreasing nPNA groups were strictly increasing, whereas a tendency toward better survival and decreased all-cause death was observed across the increasing nPNA groups, espe-

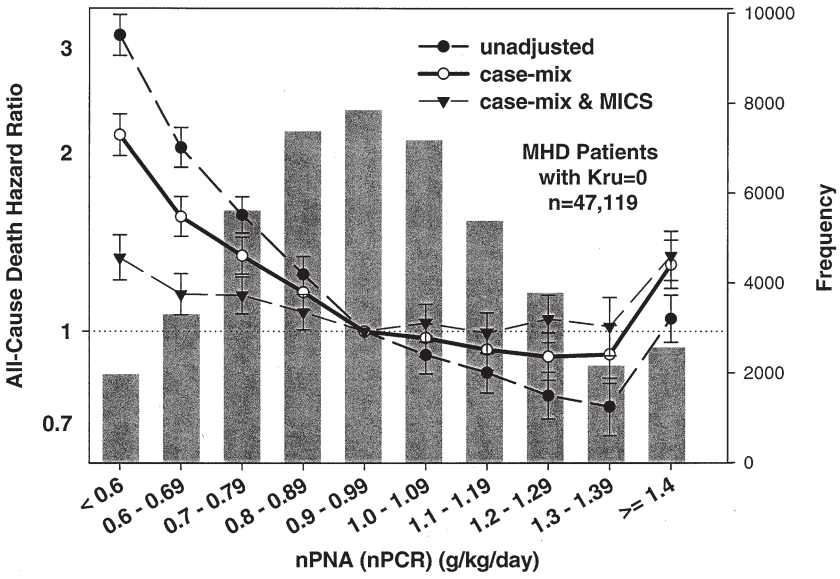

Fig 2. Association between nPNA and 2-year mortality in only the 47,119 MHD patients without significant residual renal function (urinary urea clearance = 0) using time-dependent models and quarterly varying repeated measures.

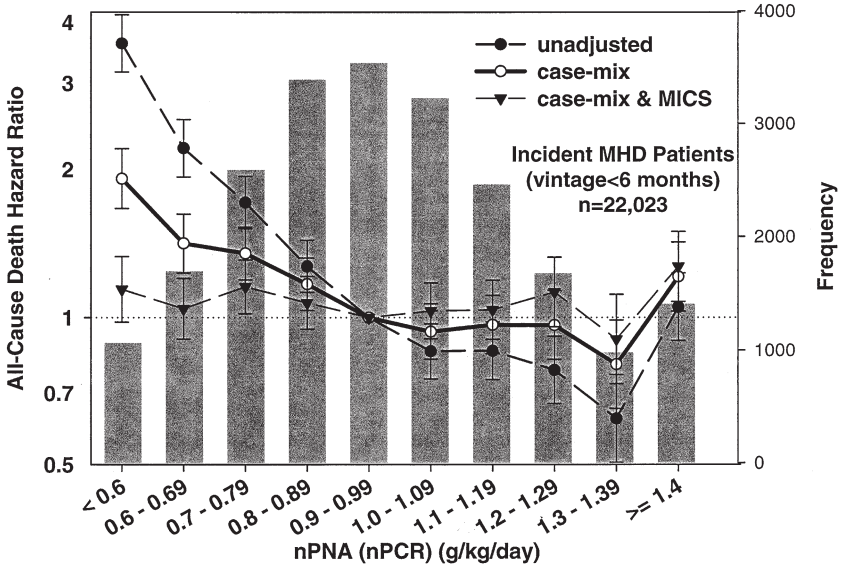

Fig 3. Association between nPNA and 2-year mortality in 22,023 incident MHD patients (dialysis vintage < 6 months) based on time-dependent models using quarterly varying repeated measures.

cially after multivariate adjustment for baseline values (Fig 5).

DISCUSSION

In this study, for the first time to our knowledge, we examine longitudinal associations between time-varying protein intake and survival in a large group of contemporary MHD patients throughout the United States. We showed that changes in protein intake during a 6-month period are predictive of prospective mortality independent of baseline nPNA or other baseline MICS markers. A decrease in nPNA over time correlated with incremental death risk independent of demographic, clinical, or other laboratory characteristics, whereas an increase in protein intake over time indicated a trend toward better survival. However, inconsistent with our primary hypothesis, we found that very high daily protein intake of 1.4 g/kg/d or greater was not associated with better survival, but, paradoxically, with a trend toward poor outcome, a so-called inverse J-shaped association. These results were derived from a contemporary national database that in-

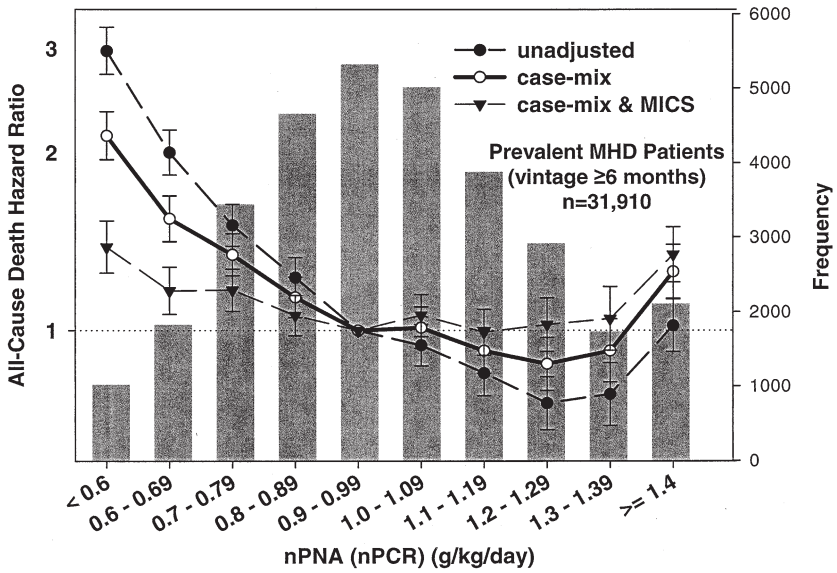

Fig 4. Association between nPNA and 2-year mortality in 31,910 prevalent MHD patients (dialysis vintage > 6 months) based on time-dependent models using quarterly varying repeated measures.

Table 5. Categories of Changes in nPNA (nPCR) During the First 6 Months in 31,587 MHD Patients Who Survived and Remained in the Cohort Through the First 6 Months of the 2-Year Cohort

Direction of Change in nPNA	Change in nPNA (g/kg/d) in q1-q2	Group Size (% of total)*	Baseline nPNA (mean ± SD)	All-Cause Death [%] During q3-q8*	CV Death [%] During q3-q8*
Decrease	≤−0.20	3,388 (11)	1.21 ± 0.26	1,072 [32]	470 [15]
	>−0.20 and ≤−0.10	4,237 (13)	1.08 ± 0.23	1,216 [29]	507 [13]
No change (stable)	>−0.10 and <0.10	14,280 (45)	0.98 ± 0.22	3,717 [26]	1,642 [12]
Increase	≥0.10 and <0.20	4,917 (16)	0.95 ± 0.21	1,240 [25]	583 [12]
	≥0.20	4,765 (15)	0.95 ± 0.24	1,304 [27]	583 [13]

NOTE. The number of deaths and crude all-cause and CV mortality rates in the subsequent 18 months are shown.
*Values in parentheses present the proportion of MHD patients in each category, and values in brackets indicate the crude death rate in the indicated group during the 2 years of observation.

cludes all eligible MHD patients of a major dialysis care provider with uniform patient management practices at the dawn of the 21st century. Moreover, all blood measurements were conducted in a single laboratory by using standardized laboratory techniques. Use of longitudinal and time-varying assessment of nutritional indices in our study may have important clinical implications, especially because physicians and dietitians usually evaluate patients by using current, rather than historical or baseline, data. Clinical evaluations usually take into account current protein intake estimates, rather than previous values at the baseline of some hypothetical cohort.

Almost two thirds of all MHD patients in the United States die within 5 years of initiation of long-term dialysis treatment, mostly of CV disease.[20] Survival in dialysis patients has not improved substantially in the past 2 decades.[21] Recent randomized clinical trials showed no survival benefit of cholesterol-lowering interventions using atorvastatin (the Die Deutsche Diabetes Dialyse Study)[22] or high-dose folic acid to treat hyperhomocysteinemia[23] in dialysis patients. Additional efforts in the form of several multicenter clinical trials failed to show a survival advantage of increasing dialysis dose in these patients.[24,25] Furthermore, observational studies showed only a modest, if any, association between hypertension and survival in dialysis patients.[8,26,27] Hence, there appears to be other prevailing conditions that contribute to this substantial and persistent CV disease and mortality rate that need to be identified and studied better. Our study reiterates that protein intake may be an important nontraditional risk factor in these patients.

Among measures of protein intake in MHD patients, net urea generation measurements, determined by using nPNA, are used most often because they are easily measurable and math-

Table 6. Association Between Change in nPNA (nPCR) During the First 6 Months and Death in the Subsequent 18 Months in Patients With a Baseline nPNA of 0.8 to 1.2 g/kg/d

All-Cause Mortality	Unadjusted		Case-Mix Adjusted		Case-Mix and MICS Adjusted	
nPCR Change	Hazard Ratio (95% confidence interval)	*P*	Hazard Ratio (95% confidence interval)	*P*	Hazard Ratio (95% confidence interval)	*P*
≤−0.20	1.58 (1.44-1.75)	<0.0001	1.61 (1.46-1.78)	<0.0001	1.48 (1.34-1.64)	<0.0001
>−0.20 and ≤−0.10	1.24 (1.14-1.35)	<0.0001	1.24 (1.14-1.35)	<0.0001	1.18 (1.08-1.28)	0.0001
>−0.10 and <0.10*	1.0	NA	1.0	NA	1.0	NA
≥0.10 and <0.20	0.96 (0.88-1.05)	0.39	0.98 (0.90-1.07)	0.59	0.96 (0.88-1.05)	0.36
≥0.20	1.03 (0.94-1.13)	0.48	1.11 (1.01-1.21)	0.03	0.97 (0.89-1.07)	0.56

NOTE. Covariates in case-mix include age, sex, race/ethnicity, diabetes mellitus, vintage, insurance, marital status, standardized mortality ratio, Kt/V, tobacco use, residual urine, and history of 10 comorbid conditions. Covariates in MICS include serum albumin, TIBC, ferritin, creatinine, calcium, phosphorus, bicarbonate, blood hemoglobin, WBCs, lymphocyte %, BMI, and administered EPO dose.
*Reference group.

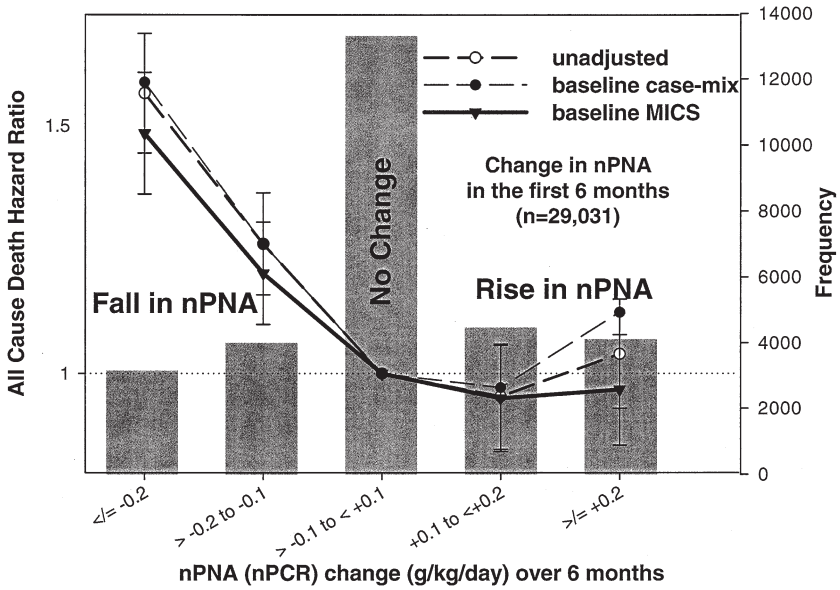

Fig 5. Association between change in nPNA (nPCR) during the first 6 months and death in the subsequent 18 months in patients with a baseline nPNA of 0.8 to 1.2 g/kg/d.

ematically accurate. nPNA, usually measured monthly in most dialysis clinics, was shown to correlate with morbidity and mortality in MHD patients.[14,28-32] Although the National Cooperative Dialysis Study (NCDS)[29,30,32] suggested that correlation between nPNA and measured dietary protein intake was weak at best, NCDS data originate from the late 1970s and may not accurately apply to the modern dialysis technique–based urea kinetics. However, even in the NCDS, nPNA greater than 1.0 g/kg/d and timed average serum urea concentration of approximately 50 mg/dL were associated with low morbidity.[29,30] A recent observational study showed that low nPNA in MHD patients with a Kt/V greater than 1.2 was associated strongly with a greater death rate and increased hospitalization.[14] Our present results are consistent with these findings because low nPNA less than 0.8 g/kg/d had a robust association with both all-cause and CV mortality, which persisted at all levels of multivariate adjustments.

There are limitations to the preciseness with which nPNA indicates daily protein intake. First, measured nPNA is dependent on the manufacturer's estimates of dialyzer permeability characteristics and also on the accuracy of measured blood and dialysate flow rates.[14] Second, there are fluctuations in nPNA from day to day caused by changes in daily protein intake or endogenous protein catabolism.[33] Third, when daily protein intake is less or greater than 1.0 g/kg/d, it may be overestimated or underestimated by using nPNA, respectively.[34] Fourth, for nPNA to accurately estimate protein intake, the patient's protein metabolism should be at equilibrium or nearly so at the time of measurement.[35] This condition is not always met, particularly in MHD patients with many comorbid conditions or acute disease states. However, our use of 3-month averaged nPNA values should mitigate this confounder. Fifth, the volume of distribution of urea may be difficult to estimate accurately, particularly in obese, malnourished, or edematous patients.[36] Sixth, delayed equilibrium with subsequent urea rebound after dialysis, which can vary according to the patient and characteristics of the dialysis procedure, may lead to an overestimate of nPNA.[37] Finally, nPNA gives a more precise estimate of measured total nitrogen output in MHD patients, rather than estimating nitrogen intake through dietary interviews.[38,39] Hence, the relationship between daily protein intake and nPNA in stable MHD patients is linear over a wide range of protein intake.[38] Thus, in large outpatient population groups, including such nation-wide epidemiological studies as ours, nPNA can be used reliably as a surrogate of dietary protein intake.

One of the reported limitations of nPNA is its mathematical coupling with dialysis dose (Kt/V).[14] In our study, the correlation coefficient between Kt/V and nPNA was +0.28. However, an alterna-

tive explanation is that the change in measured nPNA may be a consequence of a better dialysis dose and subsequently improved appetite with greater nutrient intake, leading to improved survival.[2] In a recent study, a significant association between Kt/V and nPNA was found only for Kt/V values less than 1.2.[14] All multivariate models in our study were adjusted for Kt/V; hence, it is unlikely that the mathematical or clinical association between these 2 measures had a bearing on the multivariate adjusted association between nPNA and mortality. Another potential limitation of nPNA is that the metabolic status of any given patient cannot be estimated by it; patients may be in negative or positive nitrogen balance for a variety of reasons.[40] However, MHD patients are rarely in more than 1.0 to 1.5 g/d of negative or particularly positive nitrogen balance for more than a very few days.[14,40] Thus, except for exceptional times in which an MHD patient is in strongly negative or positive nitrogen balance, nPNA should closely reflect dietary protein intake (eg, within ±10% to 15%). Moreover, the extremely large size of our study and use of the 13-week averaged nPNA in lieu of 1 single measurement mitigate the effect of nitrogen balance status on our examined associations.

In our study, there was a downward trend in mortality risk with progressively greater amounts of daily protein intake only up to nPNA of 1.4 g/kg/d. The highest nPNA group (>1.4 g/kg/d) showed a reverse J-shaped upward trend in CV death. This may be caused by the outcome-associated confounding effect of body weight in smaller patients, the toxic effect of a very high-protein diet, a highly catabolic state caused by inflammation, or reflection of a behavior pattern of poor compliance. Another notable finding is the mitigation, but not complete removal, of the protective effect of moderately high nPNA on survival upon further adjustment for MICS surrogates, leading to an almost flat line for the 1.0- to 1.4-g/kg/dL nPNA range shown in Figs 1 through 4. This may be caused by an overadjustment bias,[41,42] especially because MICS may be in the causal pathway of the association between protein intake and survival, as shown schematically in Fig 6. Hence, although adjustment for case-mix, comorbid conditions, and dialysis dose appears justified by graphical criteria,[43,44] additional multivariate adjustment for MICS may be inappropriate. Nonetheless, the observed residual association between protein intake and survival supports the presence of an effect.

In our study, an increase in protein intake over time was associated with a trend for improved all-cause death risk, but this effect was not as strong as the incremental association between decreasing protein intake and worsening death risk (Fig 5). These findings may have occurred

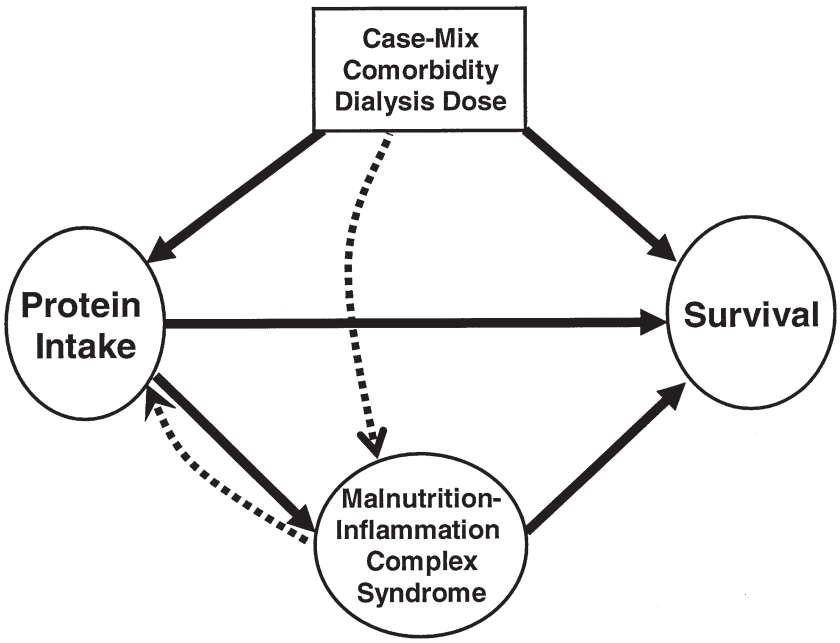

Fig 6. Hypothetical association between dietary protein intake and survival in MHD patients and the possible role of other factors. Whereas case-mix, comorbidity, and dialysis dose may be confounders, MICS may be in the causal pathway of the association between protein intake and survival.

because patients who have poor nutritional status at baseline may continue to carry some of the residual high-risk effects, even after they are able to increase their protein intake. Moreover, high nPNA or abrupt increase in nPNA may indicate a negative nitrogen balance and a catabolic state during infection, inflammation, and other disease states. Hence, it appears possible that strictly improving survival could be achieved with an increased nPNA of less than 1.4 g/kg/d or a moderate increase during a 6-month interval. Future studies should examine the cause of the reverse J-shaped association.

Our analyses also included tobacco smoking and preexisting CV and non-CV comorbid conditions obtained from the dialysis initiation form (Form 2728), history of diabetes mellitus, and many other covariates known to have strong associations with comorbid conditions. Although we lacked values for explicit laboratory markers of inflammation, such as C-reactive protein, we had values for serum albumin, ferritin, TIBC, blood WBCs, lymphocyte, and hematocrit and administered dose of EPO, all associated with inflammation.[45-49]

Among the strengths of our study is the use of time-dependent models to examine the relation of nPNA groups to CV mortality while controlling for other time-varying nutritional and inflammatory indices and dialysis dose. Our data are based on a 2-year cohort period, rather than a more extended longitudinal follow-up over many years; hence, our results may not apply to long-term survival. Nonetheless, the narrow time window of our study ensures that confounding by changes in practice or technology is minimal. Our time-dependent findings are supported by the observed relations of different rates of nPNA gain and loss over time to survival. The data originate from 1 dialysis care provider that has uniform patient management practices; all laboratory measurements are performed in 1 facility, and most data are means of several measures. Hence, measurement variability is minimized.

Our effect estimates are subject to various potential biases, not all of which are addressable with the observational database available to us. Nonetheless, if causal, the time-dependent association of nPNA and its changes over time with survival would have major clinical and public health implications. Clinical trials based on dietary interventions in MHD patients, including nutritional supplements with or without anti-inflammatory or antioxidant properties, could provide valuable information pertaining to the cause of the association between nPNA and mortality.[50] Such interventions also may include appetite-stimulating agents[51] because a poor appetite is associated with MICS and poor outcome.[2] Until trials are conducted, special caution should be exercised in interpreting such observational data as ours.

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