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Serum albumin is incrementally associated with increased mortality across varying levels of kidney function

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Highlights

- In this National Health and Examination Survey cohort of 31,274 adults, case-mix adjusted analyses suggested that participants with incrementally lower sAlb levels <4.6g/dL had increasingly higher mortality risk compared to those with sAlb ranging 4.6-<4.8g/dL (reference).
- Spline analyses showed that participants with sAlb <4.6g/dL had higher mortality across all levels of eGFR ranging from 30 to 120 ml/min/1.73m^2 (reference: sAlb ≥4.6g/dL).
- There was a graded association between lower sAlb levels with higher death risk, which may be robust across varying levels of kidney function.

Original Article

Serum albumin is incrementally associated with increased mortality across varying levels of kidney function

(Running Head: Albumin, eGFR and Survival)

Authors

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Author Statement

Amanda Brown Tortorici: Conceptualization, Formal analysis, Writing – original draft, Funding acquisition. **Elani Streja:** Conceptualization, Formal analysis. **Neda Naderi:** Writing – original draft, review & editing. **Ying Tang:** Formal analysis. **Christina Park:** Formal analysis. **Amy You:** Formal analysis. **Keith Norris:** Writing – review & editing. **Yoshitsugu Obi:** Formal analysis. **Kamyar Kalantar-Zadeh:** Conceptualization, Writing – review & editing. **Connie Rhee:** Supervision, Writing – review & editing.

Abstract

Background: Serum albumin (sAlb) may be a strong predictor of longevity in the general population and in chronic kidney disease.

Objective: To determine the relationship between sAlb concentrations and mortality risk independent of kidney function.

Methods: Retrospective cohort study of 31,274 adults from the 1999-2010 National Health and Nutrition Examination Survey. Estimated glomerular filtration rate (eGFR) was examined as both a confounder and modifier of the association of sAlb with mortality risk. We examined the association of sAlb (categorized as <3.8, 3.8-<4.0, 4.0-<4.2, 4.2-<4.4, 4.4-<4.6, 4.6-<4.8, ≥4.8g/dL) with mortality using Cox models. We then conducted spline analyses to estimate the association of sAlb with all-cause mortality across varying eGFR levels.

Results: In unadjusted analyses, participants with incrementally lower sAlb concentrations <4.6g/dL had increasingly higher mortality risk compared to those with sAlb ranging 4.6-<4.8g/dL (reference), whereas those with higher sAlb ≥4.8g/dL had lower mortality risk: HRs (95%CI) 3.88 (3.26, 4.62), 3.59 (3.01, 4.27), 2.79 (2.37, 3.29), 2.10 (1.79, 2.48), 1.72 (1.45, 2.03), and 0.71 (0.55, 0.92) for sAlb concentrations of <3.8, 3.8-<4.0, 4.0-<4.2, 4.2-<4.4, 4.4-<4.6, and ≥4.8g/dL, respectively. Adjusted analyses showed similar findings, although the association of higher sAlb ≥4.8g/dL with better survival was attenuated to the null. Spline analyses showed participants with sAlb <4.6g/dL had higher mortality across all concentrations of eGFR ranging from 30 to 120 ml/min/1.73m^2 (reference: sAlb ≥4.6g/dL).

Conclusion: Among a nationally representative US cohort, there was a graded association between lower sAlb concentrations with higher death risk, which was robust across varying levels of kidney function.

Key words:

Serum albumin, kidney function, mortality, national health and examination survey, NHANES, estimated glomerular filtration rate, eGFR

Introduction

Serum albumin (sAlb), a globular protein consisting of 585 amino acids and a molecular weight of 66.5 kilodaltons, is the most abundant protein in the blood which accounts for at least 75% of oncotic pressure due to its large molecular weight and negative charge. Albumin is synthesized in the liver and released at a rate of 10-15 grams (g) per day into the bloodstream. Serum albumin (sAlb) is measured routinely as a core component of most metabolic panels, and provides insight into patients' nutritional, inflammatory, and overall health status.

The normal reference concentrations for sAlb typically span across a wide range of 3.5 to 5.2g/dL, and lower concentrations of serum albumin <3.5g/dL have been associated with higher mortality risk in the general population, as well as those with chronic disease states. Given that protein-energy wasting is a major contributor to death in chronic kidney disease (CKD) patients, low serum albumin has been recognized as a marker of poor nutritional status and potent predictor of death in this population.(1, 2) However, the impact of sAlb within so-called normal ranges (>3.5g/dL) upon the health and survival of the general population as well as those with CKD has not been well studied, with prior studies limited by lack of granular examination of sAlb concentrations and non-consideration of kidney function as a potential confounder.(1, 2) Furthermore, no studies have examined the relationship between sAlb with mortality risk across varying levels of estimated glomerular filtration rates (eGFRs).

Hence, to address this knowledge gap, we sought to test the hypothesis that lower sAlb concentrations even within purportedly normal ranges of >3.5g/dL are associated with higher

mortality risk independent of kidney function. Using data from the 1999-2010 continuous Nutrition Health and Examination Survey (NHANES), we examined granular concentrations of sAlb with survival in the overall cohort, as well as across varying levels of kidney function.

Subjects and Methods

Source Cohort

We conducted a retrospective cohort study examining data from the 1999-2010 continuous NHANES population. The purpose of NHANES is to assess the nutritional status, health behaviors, and health status of US non-institutionalized civilians. (3) Data from participants enrolled in the continuous NHANES cohort were released in two-year cycles. From 1999-2006, oversampled populations included elderly, adolescent, Mexican-American, Non-Hispanic Black, and low-income persons. From 2007-2010, oversampled populations included those of elderly, Hispanic, and low-income background.(3)

In order to select a study cohort representative of the US non-institutionalized civilian population, a complex, multistage probability design was utilized. Within each two-year cycle, about 12,000 persons were asked to participate. Potential households were sent letters in the mail as notification of selection into the study. Initial interviews were conducted at the participants' home. The first interview served as a screening tool to determine if any or all of the household members were eligible to continue with participation. If household members were approved for participation, three additional interviews were conducted including family, sample participant, and relationship questionnaires. Following the home-based interviews, participants were requested to undergo clinical laboratory tests, physical examinations, and nutrition-related interviews located at mobile exam centers.(3)

Patients were included provided that they were 1) age ≥18 years of age, 2) had available serum albumin data, and 3) did not have missing mortality data. Patients with underlying liver disease and nutritional disorders were retained in the cohort in order to preserve generalizability.

Exposure Ascertainment

We examined the association of sAlb with all-cause mortality in the overall cohort, as well as across varying levels of kidney function. Our primary exposure of interest was sAlb concentration. Within the continuous NHANES cohort, sAlb concentration was measured using the DcX800 method with Bromcresol Purple (BCP) reagent. (3) We first examined baseline sAlb concentrations categorized as <3.8, 3.8-<4.0, 4.0-<4.2, 4.2-<4.4, 4.4-<4.6, 4.6-<4.8, ≥4.8g/dL. We then examined continuous sAlb concentrations using restricted cubic spline analyses.

We also examined estimated glomerular filtration rate (eGFR) as both a key confounder and modifier of the association of sAlb with mortality risk. The concentration of serum creatinine was established using the Jaffe rate method (kinetic alkaline picrate). (3) To adjust for errors in the 1999-2000 cohort creatinine measurements, the Deming regression was used: Standard Creatinine (Y) = 1.013*NHANES Creatinine (X) + 0.147 (r = 0.984).(3) In the 2005-2006 cohort creatinine measurements, the following equation was used to adjust for errors: Standard creatinine (mg/dL) = -0.016 + 0.978 * (NHANES 05-06 uncalibrated serum creatinine, mg/dL). (3) The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to estimate GFR. (4)

Outcome Ascertainment

All-cause mortality was the primary outcome of interest. Mortality data was obtained through the National Center for Health Statistics (NCHS) Public-Use Linked Mortality Files. (5)

Mortality data was collected starting from the date of NHANES participation through the end of December 31, 2011. Participant at-risk time began the day after sAlb measurement and ended at death or end of the follow-up period.

Statistical Analyses

Participants' baseline characteristics were summarized using means ± standard deviations (SDs) and proportions according to sAlb category (<4.2, 4.2-<4.6, ≥4.6g/dL) and eGFR (<60, 60-<90, ≥90ml/min/1.73m^2). Kaplan-Meier plots and log-rank testing were first conducted to ascertain unadjusted associations between sAlb concentrations and mortality. Participants were defined to be at-risk of death from the day after sAlb measurement until death or end of follow-up. sAlb concentrations were divided into seven categories (<3.8, 3.8-<4.0, 4.0-<4.2, 4.2-<4.4, 4.4-<4.6, 4.6-<4.8, ≥4.8g/dL) to evaluate the association between sAlb concentrations and all-cause mortality using 4.6-<4.8g/dL as the reference group. Associations of sAlb concentrations with all-cause mortality risk were examined using the following levels of adjustment (6):

(1) *Unadjusted model*: Included sAlb concentration;
(2) *Case-mix adjusted model*: Included covariates in the unadjusted model, as well as age, sex, race/ethnicity, level of education, diabetes, smoking status, systolic blood pressure, and serum total cholesterol level;
(3) *Case-mix + eGFR adjusted model*: Included covariates in the case-mix model, as well as eGFR.

We a priori defined the case-mix adjusted model as our primary model, which included potential confounders of the sAlb—mortality association. We designated the case-mix + eGFR

adjusted model as an exploratory model, given that eGFR may be interpreted as a potential confounder vs. intermediate of the sAlb—mortality relationship (i.e., in terms of the former, kidney dysfunction may influence appetite and thereby influence nutritional/protein intake; in terms of the latter, higher protein intake may lead to kidney function deterioration in the context of CKD). As sAlb is also a marker of inflammation (i.e., negative acute phase reactant), we also implemented sensitivity analyses in which we incrementally adjusted for C-reactive protein (CRP) using case-mix + eGFR + CRP adjusted models.

Subgroup analyses were conducted to explore associations of lower sAlb (<4.6g/dL) vs. higher sAlb concentrations (≥4.6g/dL) with all-cause mortality across strata of selected subgroups including: age (<46 vs. ≥46 years), sex (male vs. female), race/ethnicity (Non-Hispanic White vs. Non-Hispanic Black vs. Hispanic), self-reported diabetes status (diabetic vs. borderline diabetic vs. non-diabetic, in response to the question "Other than during pregnancy, have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?"), smoking status (non-smoking vs. every day vs. some days), systolic blood pressure (<120mmHg vs. ≥120mmHg), total cholesterol level (<200 vs. ≥200mg/dL), and eGFR (<60 vs. 60-<90 vs. ≥90 ml/min/1.73m^2).

We also examined the differential association of high (≥ 4.6mg/dL) vs. low (<4.6g/dL) sAlb concentration with all-cause mortality across varying eGFR levels examined as a continuous variable. Three restricted cubic spline functions were created for eGFR with four knots at the 5th, 35th, 65th, and 95th percentiles. We then included the dichotomized sAlb variable, spline functions of eGFR, and their interaction terms in the Cox model along with the aforementioned covariates. Hazard ratios (HRs) of high vs. low sAlb at a given eGFR level were estimated as follows:

HR of high serum albumin = $\beta_{alb} + \beta_1 \times SP_1 + \beta_2 \times SP_2 + \beta_3 \times SP_3$

where SP_1, SP_2, and SP_3 are values of spline functions at a given eGFR, respectively; β_{alb} is the coefficient of the dichotomized sAlb; and β_1, β_2, and β_3 are the coefficients of the interaction terms of SP_1, SP_2, and SP_3 with the dichotomized sAlb, respectively.

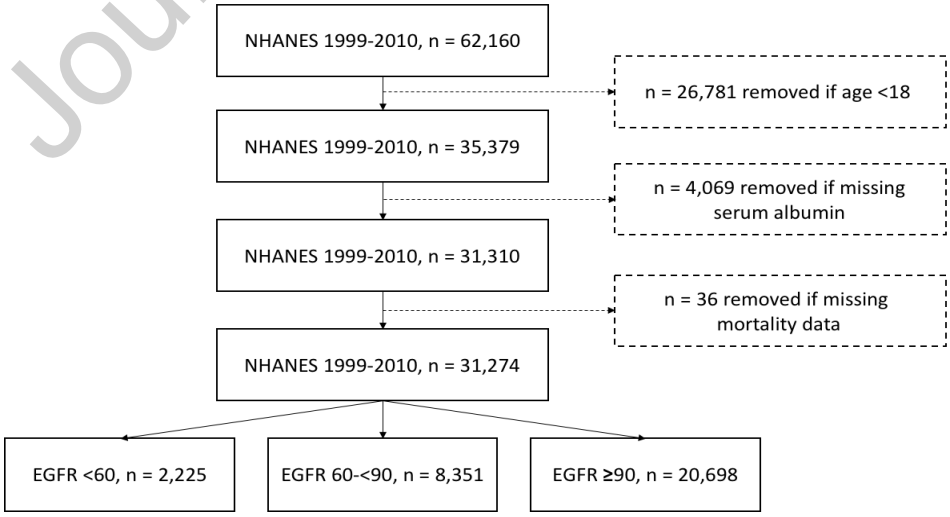
Imputation by means was used to account for missing data. Total cholesterol had <0.01% (N=3) missing values, while systolic blood pressure had 54.7% (N=17,122) missing values. All other covariates had complete data. Spline analyses were conducted using Stata version 13.1 (Stata Corporation, College Station, Texas). All other analyses were carried out using SAS 9.4 (SAS Institute, Inc., Car, North Carolina).

Results

Study Population

After applying eligibility criteria, the final study cohort was comprised of 31,274 participants (**Figure 1**), among whom 11,384 participants had sAlb concentrations <4.2g/dL, 13,834 participants had sAlb concentrations 4.2-4.6g/dL, and 6,056

Figure 1.



Study flow chart.

participants had sAlb concentrations >4.6g/dL. The mean±SD and median sAlb concentrations of the overall cohort were 4.2±0.4g/dL and 4.3g/dL, respectively. Baseline characteristics stratified by sAlb category are shown in **Table 1**. Compared to participants with lower sAlb

Table 1. Baseline characteristics of National Health and Nutrition Examination Survey participants (N=31,274) according to serum albumin categories.

	Serum Albumin (g/dL)				
	Total	**<4.2**	**4.2-4.6**	**≥4.6**	**p-value**
N (%)	31,274	11384 (36.4)	13834 (44.2)	6056 (19.4)	
Age (mean+SD)	48±20	51±20	48±19	39±18	<0.001
Female (%)	52	68	48	29	<0.001
Race (%)					<0.001
Non-Hispanic White	48	45	49	50	
Non-Hispanic Black	20	26	18	12	
Hispanic	28	26	28	33	
Other	4	4	4	5	
Education (%)					
< 9th grade	13	14	13	11	<0.001
9-11th grade	19	19	18	19	
High school/GED	24	24	24	26	
Some college	26	27	26	25	
College graduate	18	16	18	19	
Refused	<0.1	<0.1	<0.1	<0.1	
Don't know	<0.1	<0.1	<0.1	<0.1	
Smoking status (%)					
Every day	17	17	17	17	<0.001
Some days	3	3	3	5	
Not at all	24	25	25	19	
Diabetes (%)	10	14	9	6	<0.001
Systolic BP, mmHg (mean±SD)	124±21	126±23	125±21	121±17	<0.001
Total cholesterol, mg/dL (mean±SD)	197±43	197±45	198±42	194±43	0.005
eGFR, ml/min/1.73m^2 (mean±SD)	97±26	95±29	96±24	105±23	<0.001

Abbreviations: GED, General Education Development; BP, blood pressure; eGFR, estimated glomerular filtration rate.

concentrations (<4.2g/dL), those with higher sAlb concentrations (≥4.6g/dL) tended to be younger and male; had a higher prevalence of Non-Hispanic White and Hispanics and a lower prevalence of Non-Hispanic Blacks; were more likely to be college graduates; were more likely to be non-diabetic and less likely to be non-smokers; had lower systolic blood pressure values; and had lower total cholesterol and higher eGFR levels. Baseline characteristics stratified by eGFR category are shown in **Table 2**.

Table 2. Baseline characteristics of National Health and Nutrition Examination Survey participants (N=31,274) according to baseline estimated glomerular filtration rate category.

	Estimated GFR ml/min/1.73m^2				
	Total	**< 60**	**60 - <90**	**≥ 90**	**p-value**
N (%)	31,274	2681 (8.6)	8923 (28.5)	19670 (62.9)	
Age (mean+SD)	48±20	74±10	61±15	38±15	<0.001
Female (%)	52	53	47	54	<0.001
Race (%)					<0.001
Hispanic	28	13	19	35	
White	48	67	62	39	
Black	20	17	15	22	
Other	4	3	4	5	
Education (%)					0.38
< 9th grade	13	20	14	12	
9-11th grade	19	17	14	21	
High School/GED	24	25	24	24	
Some college	26	21	26	27	
College grad	18	16	22	16	
Refused	0.05	0.22	0.04	0.03	
Don't know	0.11	0.19	0.17	0.07	
Smoking status (%)					<0.001
Every day	17	8	14	19	
Some days	3	1	2	4	
Not at all	24	41	34	17	
Diabetes (%)	10	26	13	6	<0.001
Systolic BP, mmHG (mean±SD)	124±21	141±26	132±22	119±17	<0.001

Total cholesterol, mg/dL (mean±SD)	197±43	195±46	203±42	194±43	<0.001
Serum albumin concentration, g/dL (mean±SD)	4.24±0.38	4.09±0.36	4.23±0.31	4.27±0.40	<0.001
Proportion with albumin <4.2g/dL	36	53	38	33	
Proportion with albumin ≥4.6g/dL	19	7	14	23	

Abbreviations: GED, General Education Development; BP, blood pressure; eGFR, estimated glomerular filtration rate.

Predictors of Serum Albumin Concentration

In adjusted logistic regression analyses, significant predictors of lower sAlb concentrations <4.6g/dL included older age, female sex, Non-Hispanic Black race/ethnicity, and presence of diabetes (**Table 3**). In contrast, significant predictors of higher sAlb concentrations ≥4.6g/dL included Hispanic race/ethnicity and having completed college.

Table 3. Logistic regression analyses of predictors of serum albumin concentrations <4.6g/dL (reference: ≥4.6g/dL) among National Health and Nutrition Examination Survey participants (N=31,274).

	Unadjusted			Adjusted		
Variable	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Age (per 10 years)	1.34	(1.32-1.37)	<0.001	1.39	(1.35-1.43)	<0.001
Female (vs. Male)	3.33	(3.13-3.54)	<0.001	3.97	(3.63-4.35)	<0.001
Race (%)						
White	Ref.			Ref.		
Hispanic	0.88	(0.82-0.93)	<0.001	0.85	(0.77-0.95)	<0.001
Black	1.9	(1.74-2.07)	<0.001	2.39	(2.09-2.73)	<0.001
Other	0.86	(0.75-0.98)	<0.001	1.15	(0.92-1.44)	0.38
Education (%)						
Some college	Ref.			Ref.		
<9th grade	1.26	(1.14-1.39)	0.01	1.04	(0.89-1.22)	0.42
9-11th grade	0.93	(0.85-1.01)	0.92	1	(0.88-1.14)	0.54
High school graduate/GED	0.94	(0.87-1.02)	0.99	1.04	(0.93-1.17)	0.41
College graduate	0.89	(0.81-0.97)	0.60	0.87	(0.77-1.00)	0.96
Smoking status (%)						

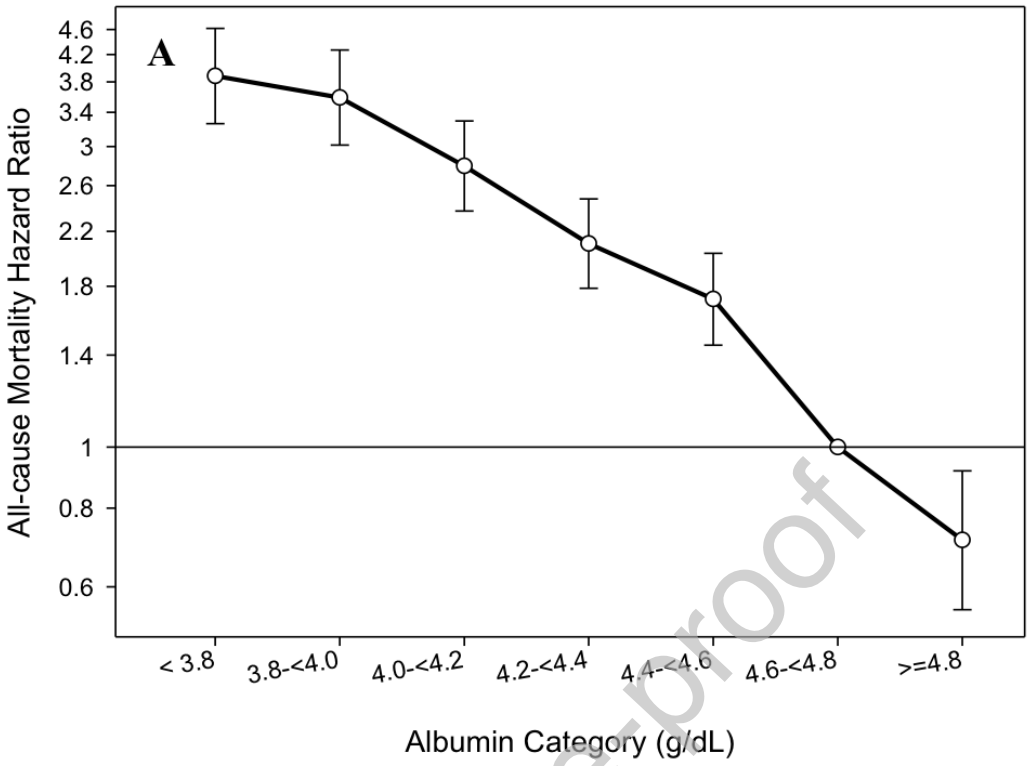
Never smoke	Ref.			Ref.		
Every day	0.79	(0.72-0.86)	0.09	1	(0.87-1.15)	0.06
Some days	0.53	(0.46-0.62)	<0.001	0.79	(0.63-0.99)	0.09
Diabetes	2.43	(2.14-2.74)	0.01	1.43	(1.19-1.73)	0.23
Systolic BP (per 30 mmHg)	1.42	(1.34-1.51)	<0.001	0.89	(0.82-0.97)	0.007
Total cholesterol (per 100 mg/dL)	1.18	(1.10-1.26)	<0.001	1.02	(0.92-1.12)	0.75
eGFR (per 10ml/min/1.73m^2)	0.865	(0.86-0.88)	<0.001	0.954	(0.94-0.97)	<0.001

Abbreviations: GED, General Education Development; BP, blood pressure; eGFR, estimated glomerular filtration rate.

Serum Albumin Concentration and Mortality

In the overall cohort, a total of 2,803 all-cause deaths were observed. Median (IQR) follow up time was 73 (39, 112) months. In unadjusted analyses, we observed that incrementally lower sAlb concentrations <4.6g/dL were associated with higher death risk (reference: 4.6-<4.8g/dL), whereas higher sAlb concentrations ≥4.8g/dL were associated with lower death risk (**Figure 2A and Table 4**): HRs (95%CI) 3.88 (3.26, 4.62), 3.59 (3.01, 4.27), 2.79

Figure 2A.



Association between serum albumin and all-cause mortality in an unadjusted model among National Health and Nutrition Examination Survey participants (N=31,274).

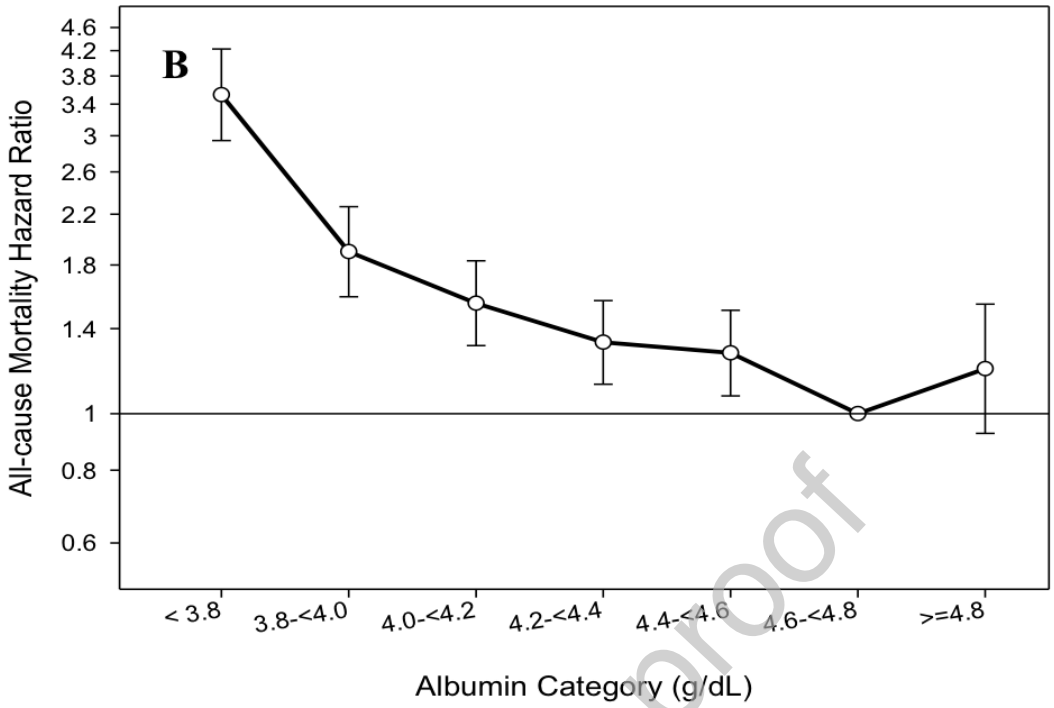
Table 4. Association between serum albumin and all-cause mortality in unadjusted, case-mix, and case-mix + estimated glomerular filtration rate + C-reactive protein models among National Health and Nutrition Examination Survey participants (N=31,274).

Serum Albumin (g/dL)	Unadjusted	Case-mix	Case-mix + eGFR	Case-mix + eGFR + CRP	Number of Deaths	Number of Participants
<3.8	3.88 (3.26, 4.62)	3.53 (2.04, 4.23)	3.20 (2.67, 3.84)	2.96 (2.47, 3.57)	400	2656
3.8-<4.0	3.59 (3.01, 4.27)	1.90 (1.59, 2.27)	1.80 (1.51, 2.15)	1.75 (1.47, 2.10)	403	3133
4.0-<4.2	2.79 (2.37, 3.29)	1.55 (1.31, 1.83)	1.49 (1.26, 1.76)	1.46 (1.23, 1.73)	596	5595
4.2-<4.4	2.10 (1.79, 2.48)	1.33 (1.12, 1.56)	1.27 (1.07, 1.50)	1.26 (1.06, 1.48)	627	7275
4.4-<4.6	1.72 (1.45, 2.03)	1.27 (1.07, 1.51)	1.24 (1.04, 1.47)	1.23 (1.04, 1.46)	501	6559
4.6-<4.8	Ref	Ref	Ref	Ref	188	3864
≥4.8	0.71 (0.55, 0.92)	1.19 (0.93, 1.54)	1.15 (0.89, 1.48)	1.15 (0.89, 1.49)	88	2192

(2.37, 3.29), 2.10 (1.79, 2.48), 1.72 (1.45, 2.03), and 0.71 (0.55, 0.92) for sAlb concentrations <3.8, 3.8-<4.0, 4.0-<4.2, 4.2-<4.4, 4.4-<4.6, and ≥4.6g/dL, respectively.

Following adjustment for case-mix covariates, we again observed that sAlb concentrations <4.6g/dL were associated with higher death risk; however, associations of higher sAlb concentrations ≥4.8g/dL with lower mortality were attenuated to the null (**Figure 2B** and **Table 4**): adjusted HRs (aHRs) (95% CI) 3.53 (2.04, 4.23), 1.90 (1.59, 2.27), 1.55 (1.31, 1.83), 1.33 (1.12, 1.56), 1.27 (1.07, 1.51), and 1.19 (0.93, 1.54) for sAlb concentrations <3.8, 3.8-<4.0, 4.0-<4.2, 4.2-<4.4, 4.4-<4.6, and ≥4.6g/dL, respectively.

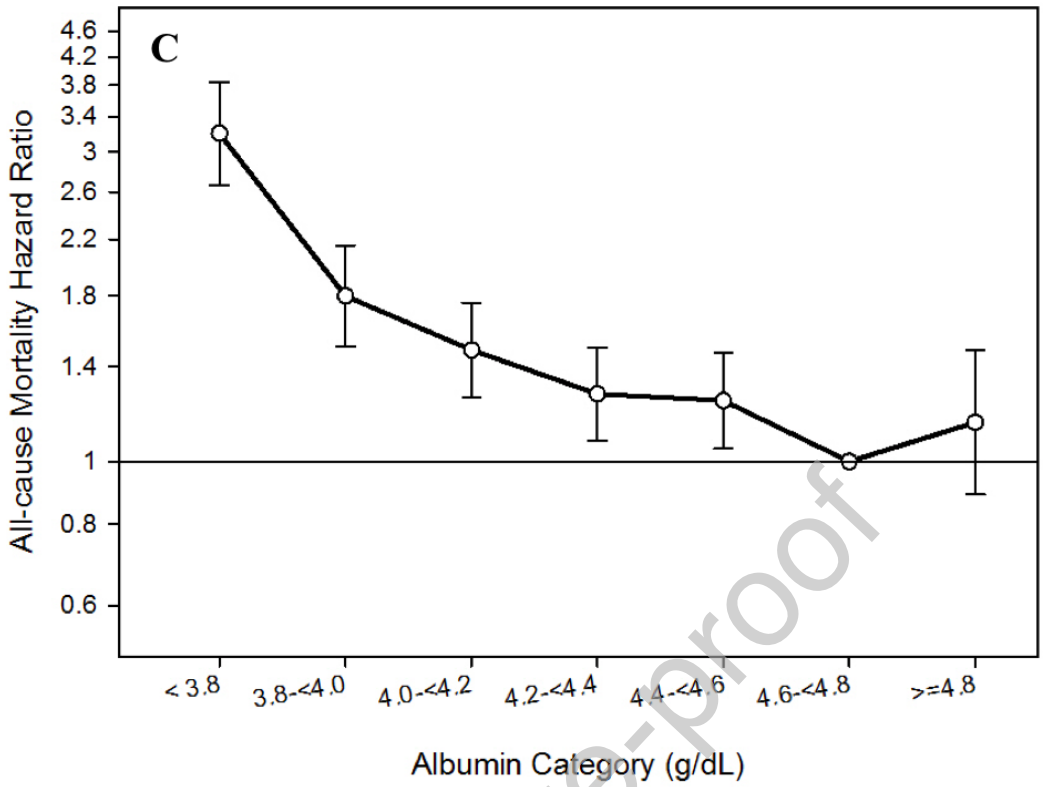
Figure 2B.



Association between serum albumin and all-cause mortality in a case-mix model among National Health and Nutrition Examination Survey participants (N=31,274).

A similar pattern of findings was observed in case-mix + eGFR and case-mix + eGFR + CRP adjusted analyses (**Figure 2C** and **Table 4**).

Figure 2C.

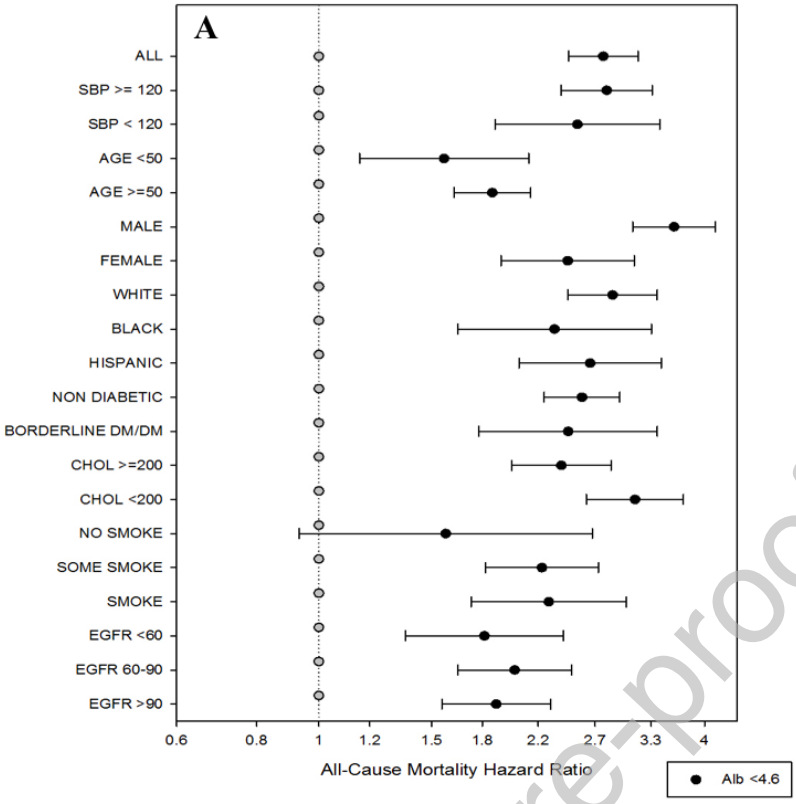


Association between serum albumin and all-cause mortality in a case-mix + estimated glomerular filtration rate model among National Health and Nutrition Examination Survey participants (N=31,274).

Subgroup Analyses

In unadjusted analyses, we observed that sAlb concentrations <4.6g/dL were associated with higher mortality risk across all subgroups, except non-smokers (reference: sAlb ≥4.6g/dL; **Figure 3A** and **Table 5**). Similarly,

Figure 3A.



Subgroup analyses of the association between serum albumin concentration <4.6 (reference: ≥4.6g/dL) with all-cause mortality in an unadjusted model among National Health and Nutrition Examination Survey participants (N=31,274).

Table 5. Subgroup analyses of the association between serum albumin concentration <4.6g/dL (reference: ≥4.6g/dL) with all-cause mortality in unadjusted (Panel A) and case-mix (Panel B) models among National Health and Nutrition Examination Survey participants (N=31,274).

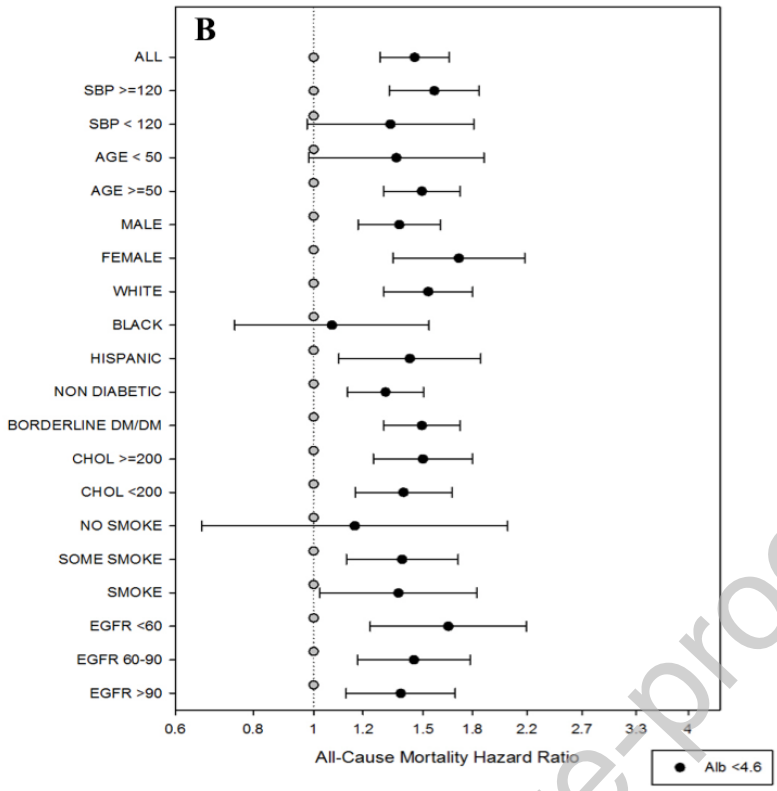
	Unadjusted		Case-mix Adjusted	
	Hazard Ratio	**95% Confidence Interval**	**Hazard Ratio**	**95% Confidence Interval**
Systolic BP < 120 mmHg	2.53	(1.88-3.41)	1.33	(0.98-1.81)
Systolic BP ≥120 mmHg	2.81	(2.39-3.31)	1.56	(1.32-1.85)
Age ≥50 years old	1.87	(1.63-2.14)	1.49	(1.30-1.72)
Age <50 years old	1.57	(1.16-2.13)	1.36	(0.98-1.88)
Female	2.45	(1.93-3.11)	1.71	(1.34-2.18)
Male	3.58	(3.09-4.16)	1.37	(1.18-1.60)

Race, Hispanic	2.65	(2.05-3.42)	1.43	(1.10-1.86)
Race, Black	2.33	(1.65-3.31)	1.07	(0.75-1.53)
Race, White	2.87	(2.44-3.37)	1.53	(1.30-1.80)
Borderline Diabetes/Diabetes	2.45	(1.78-3.38)	1.49	(1.30-1.72)
Non-Diabetic	2.58	(2.25-2.95)	1.30	(1.13-1.50)
Total Cholesterol <200 mg/dL	3.12	(2.62-3.71)	1.40	(1.16-1.67)
Total Cholesterol ≥200 mg/dL	2.39	(2.00-2.86)	1.50	(1.25-1.80)
Smoke everyday	2.29	(1.73-3.02)	1.37	(1.02-1.83)
Smoke some days	2.23	(1.82-2.73)	1.39	(1.13-1.71)
Smoke not at all	1.58	(0.93-2.67)	1.16	(0.66-2.05)
eGFR ≥90ml/min/1.73m^2	1.89	(1.56-2.30)	1.38	(1.13-1.69)
eGFR 60-<90ml/min/1.73m^2	2.02	(1.65-2.48)	1.45	(1.18-1.79)
eGFR <60ml/min/1.73m^2	1.81	(1.37-2.41)	1.65	(1.23-2.20)
CRP <1.0mg/dL	2.55	(2.22-2.94)	1.32	(1.14-1.53)
CRP ≥1.0mg/dL	2.18	(1.20-3.98)	1.60	(0.86-2.97)

Abbreviations: BP, blood pressure; eGFR, estimated glomerular filtration rate; CRP, C-Reactive Protein

in case-mix adjusted models, we observed that sAlb concentrations <4.6g/dL were associated with higher mortality risk across all subgroups, except for those younger than 50 years of age, Non-Hispanic Blacks, and with systolic blood pressure <120mmHg (**Figure 3B** and **Table 5**). In sensitivity analyses stratified by CRP level, unadjusted analyses

Figure 3B.



showed that lower sAlb—higher mortality associations were present in both those with low (<1.0mg/dL) vs. elevated CRP levels (≥1.0mg/dL); however, upon adjustment for case-mix covariates, associations persisted in those with low CRP but were attenuated to the null in those elevated CRP levels (**Table 5**).

In both unadjusted and case-mix adjusted spline analyses that examined the association of sAlb as a continuous variable and mortality risk across varying levels of eGFR, we observed that sAlb concentrations <4.6g/dL were associated with higher death risk across the entire spectrum of kidney function (reference: sAlb ≥4.6g/dL; **Figures 4A and 4B**).

Figure 4A.

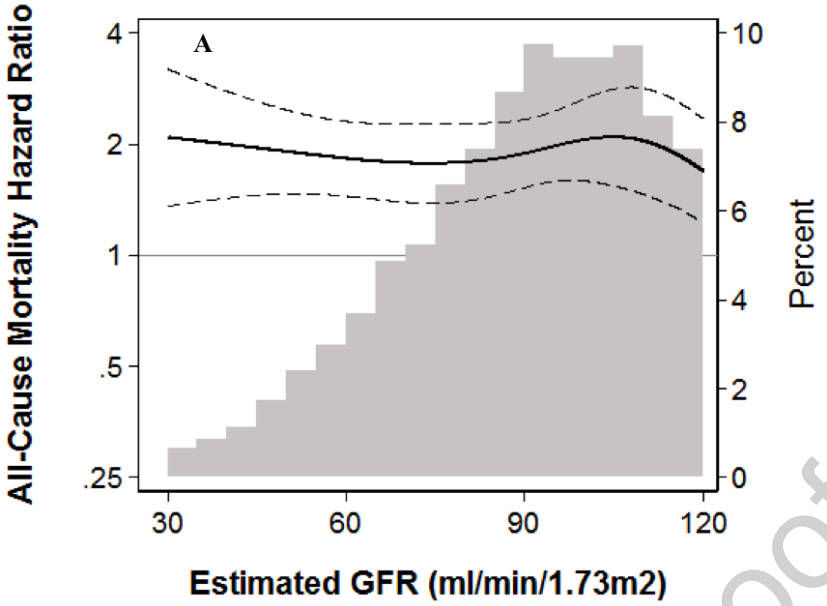
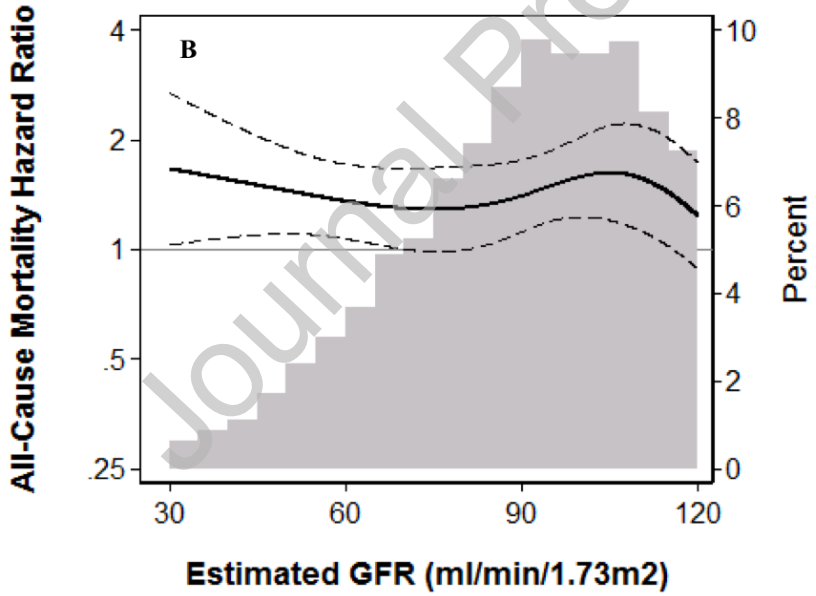


Figure 4B.



Figures 4A and 4B. Association between low serum albumin concentrations <4.6g/dL (reference: serum albumin ≥4.6g/dL) and all-cause mortality across varying estimated glomerular filtration levels in unadjusted (Panel A) and case-mix adjusted (Panel B) models among National Health and Nutrition Examination Survey participants (N=24,367 participants with eGFR ranging from 30 to 120ml/min/1.73m^2) included.

Discussion

In this nationally representative sample of US adults, crude analyses showed that incrementally lower sAlb concentrations <4.6g/dL were associated with increasingly higher death risk, whereas higher sAlb concentrations ≥4.8g/dL were associated with greater survival. After accounting for differences in case-mix covariates, eGFR, and inflammatory markers (i.e., CRP), we observed a consistent and potent association between lower sAlb concentrations and death, such that sAlb concentrations in the lowest category (i.e., sAlb <3.8g/dL, typically considered to be within the "normal" range) were associated with a two- to three-fold higher death risk; however associations between higher sAlb ≥4.8g/dL and mortality were attenuated to the null. Upon examining the association of sAlb concentrations with mortality across relevant clinical characteristics, we observed robust relationships across most subgroups. Notably, lower sAlb—higher mortality associations were observed across the entire spectrum of eGFR.

Our findings are consistent with other studies examining the relationship between sAlb and mortality in the general population. In a seminal study of the NHANES I cohort, sAlb concentrations ≥4.5g/dL compared to ≤4.1g/dL were associated with greater survival in White and Black men, as well as among women aged 45 to 74 years.(1) Analyses of the Non-Hispanic White Framingham Offspring Study cohort similarly concluded that sAlb concentrations <4.5g/dL compared to ≥4.7g/dL were associated with an increased all-cause mortality risk in women.(7) In a more recent study of over 1.7 million insurance applicants from a healthy adult population, sAlb concentrations were examined within separate age and sex strata such that the middle 50% of sAlb values specific to each group served as the reference.(8) For each age and sex strata, sAlb concentrations lower than the middle 50% of sAlb values for the respective groups were associated with higher mortality risk.

Similar to the aforementioned studies, we observed a strong association between lower sAlb concentrations in the "normal" range (<4.6g/dL) with higher mortality risk in the overall

cohort. However, to our knowledge, ours is the first study to observe a robust association between lower sAlb concentrations <4.6g/dL with higher mortality risk across the entire spectrum of kidney function examined in both categorical analyses and as a continuous spline, including those with lower levels of eGFR <60ml/min/1.73m^2. Patients with CKD are particularly predisposed to lower circulating albumin concentrations, owing to alterations in albumin synthesis/turnover resulting from concurrent illnesses, inflammation, and metabolic acidosis.(9) Indeed, several seminal studies in the CKD population have observed a strong relationship between lower sAlb and higher mortality risk, particularly below sAlb thresholds of ~3.5g/dL and 4.0g/dL.(10-12) In a meta-analysis pooling 38 studies of 265,330 dialysis-dependent CKD patients, a significant inverse association between sAlb and all-cause and cardiovascular mortality has also been observed.(13) These observations have informed clinical practice guidelines which advise maintenance of sAlb values of ≥4.0g/dL in those with advanced CKD (i.e., stage 5) patients.(11, 14) In addition, the International Society of Renal Nutrition and Metabolism has included sAlb concentrations of <3.8g/dL as one of three biochemical diagnostic criteria for protein–energy wasting.(15) However, our data suggests that among US adults with moderate CKD (i.e., eGFR <60ml/min/1.73m^2), sAlb concentrations typically considered to be in the "normal" range (i.e., sAlb <4.6g/dL) are linked with higher death risk, and that a more appropriate sAlb target may fall within ~4.6 to 4.8g/dL. Notably, while our case-mix adjusted analyses in the overall cohort demonstrated a survival benefit with sAlb concentrations >4.8g/dL, following case-mix + eGFR adjustment these associations were attenuated to the null. Given that some experts advise lower dietary protein consumption as a means to ameliorate CKD progression based on existing evidence,(16-19) we would indeed caution against using higher protein consumption as a means to achieve higher sAlb concentrations in those with CKD. Further studies are needed to confirm the optimal sAlb target across granular levels of kidney

function, and to determine safe and effective interventions that can elevate sAlb concentrations in those with CKD.

Another notable finding of our study was our observation of a differential association between sAlb and mortality across racial/ethnic groups. Among other analyses of the general population examining sAlb and mortality, Djousse et al.'s study(7) included only Non-Hispanic White participants, and while the study by Fulk et al.(8) examined age- and sex-specific sAlb references, differential race/ethnicity thresholds were not considered. In addition, although the analysis of the NHANES I cohort by Gillum et al. found a similar relationship between sAlb and mortality among Blacks and Whites, at that time the NHANES I cohort did not separately distinguish participants according to Hispanic ethnicity.(1) In our study, while Non-Hispanic Blacks had a higher likelihood of having low sAlb concentrations compared to Non-Hispanic Whites, they did not demonstrate a higher risk of mortality associated with sAlb concentrations <4.6g/dL. At this time, further studies are needed to determine the factors contributing to variations in sAlb concentrations across racial/ethnic groups, as well as their optimal sAlb targets.

The strengths of our study include its large sample size, granular examination of eGFR values, consideration of different racial/ethnic groups, and extended follow-up to observe outcomes. However, several limitations of our study bear mention. First, given that sAlb was measured at a single-point-in-time (i.e., baseline measurement only), we did not have repeated measures of sAlb as a proxy of nutritional and inflammatory status over time among participants. Hence, further studies examining the relationship between longitudinal sAlb concentrations and mortality and mortality in the general and CKD populations are needed. Second, socio-demographics and comorbidities (e.g., education, smoking status, diabetes status) were based

upon participants' self-report. Finally, the observational nature of this study prohibits causal inferences, and unmeasured confounders may have led to biased results.

In conclusion, among a cohort of healthy US adults, we observed that lower sAlb concentrations even in normal range (<4.6g/dL) are associated with higher risk of all-cause mortality independent of case-mix characteristics and eGFR. Furthermore, we observed a robust association between lower sAlb concentrations <4.6g/dL with lower survival across a broad spectrum of eGFR levels. At this time, further studies are needed to determine safe and effective interventions that can elevate sAlb concentrations in patients with and without CKD, and whether such interventions can improve survival in these populations.

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