Kidney Medicine

Proneurotensin/Neuromedin N and Risk of Incident CKD and Other Kidney Outcomes in Community-Living Individuals: The REGARDS Study

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Rationale & Objective: Plasma proneurotensin/neuromedin N (pro-NT/NMN) is a precursor of neurotensin, a tridecapeptide linked with type 2 diabetes mellitus and other comorbid conditions associated with kidney disease. Whether pro-NT/NMN is directly associated with incident chronic kidney disease (CKD), and whether that association differs by race, is uncertain. We evaluated whether pro-NT/NMN levels were associated with increased risk of kidney outcomes.

Study Design: Prospective cohort.

Setting & Participants: Participants in Biomarker Mediators of Racial Disparities in Risk Factors, a nested cohort from the REasons for Geographic And Racial Differences in Stroke study, with available stored serum and urine samples from baseline and second visits for biomarker measurement.

Exposure: Baseline log-transformed pro-NT/NMN.

Outcomes: Incident CKD, progressive estimated glomerular filtration rate (eGFR) decline, incident albuminuria, and incident kidney failure within median follow-up time of 9.4 years.

Analytical Approach: Logistic regression.

Results: Among 3,914 participants, the mean ± SD age was 64 ± 8 (SD) years, 48% were women, and 51% were Black. Median baseline eGFR was 90 (IQR, 77-102) mL/min/1.73 m^2. Each SD higher of pro-NT/NMN was associated with 9% higher odds of progressive eGFR decline (OR, 1.09; 95% CI, 1.00-1.20). There was no association observed with incident CKD (OR, 1.10; 95% CI, 0.96-1.27), incident albuminuria (OR, 1.08; 95% CI, 0.96-1.22), or incident kidney failure (OR, 1.10; 95% CI, 0.83-1.46). There were no differences in results by race or sex.

Limitations: Single measurement of pro-NT/NMN and limited generalizability.

Conclusions: Higher pro-NT/NMN was associated with progressive eGFR decline but no other manifestations of kidney disease incidence.

Complete author and article information provided before references.

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The burden of chronic kidney disease (CKD) is increasing worldwide[1] and may come to account for more than 3.1 million annual deaths by 2040.[2] Despite this critical population health issue, risk-stratification paradigms for CKD are limited largely to proxies of glomerular filtration that have limited predictive value.[3] Furthermore, early-stage diagnosis of CKD is imperative because progression to kidney failure may be halted or slowed with guideline-directed therapies for its various etiologies.[4] Identification of novel biomarkers of impending kidney dysfunction before loss of glomerular filtration function is important.

Neurotensin (NT) is a tridecapeptide[5] that appears to exert a paracrine function in the brain and heart and acts as a systemic hormone when released from the gastrointestinal tract or adrenal glands. Through measurement of its stable precursor proneurotensin/neuromedin N (pro-NT/NMN),[6] NT and its related peptides have been linked to risk of disease processes related to kidney disease, including cardiovascular disease,[7-10] obesity,[11] and type 2 diabetes mellitus.[8,9,12,13] Although NT could affect CKD through these other disease processes, NT receptors are present in the kidney,[14] and alterations in kidney physiology in response to NT infusion in humans has been reported,[15] offering the possibility that NT could directly affect kidney function.

In this study, we evaluated the association of pro-NT/NMN with incident CKD, estimated glomerular filtration rate (eGFR) decline, incident albuminuria, and incident kidney failure among participants in the REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort. Because kidney diseases are more common in Black Americans, we also evaluated the association between pro-NT/NMN among White and Black participants.[16]

METHODS

Study Design and Participants

The REGARDS study is a population-based cohort of individuals aged 45 years and older, recruited to study the reasons for the higher stroke mortality noted among Black versus White adults and in the Southeast region of the United States.[17,18] Between January 2003 and June 2007, a total of 30,239 non-Hispanic Black and White American adults were recruited. Among the exclusion criteria were race other than Black or White, active treatment of cancer, medical conditions that would prevent long-term participation, residence in or inclusion on a waiting list for a nursing home, or inability to communicate in English. Potential

PLAIN-LANGUAGE SUMMARY

Neurotensin is a peptide secreted by the small intestine in response to a meal. Higher levels of neurotensin and its stable precursor, proneurotensin/neuromedin N (pro-NT/NMN), have been associated with cardiovascular disease and type 2 diabetes mellitus, important risk factors for the development of kidney disease. Whether pro-NT/NMN is directly associated with kidney outcomes has been less studied and has been done so in largely homogenous cohorts of White participants. Using the REasons for Geographic And Racial Differences in Stroke study, we followed Black and White participants and evaluated the risk of developing kidney outcomes. We found that elevated levels of pro-NT/NMN were associated with kidney function decline. Pro-NT/NMN may help individuals who may benefit from closer monitoring of kidney function.

participants were contacted by mail with a subsequent computer-assisted telephone interview; an in-home visit for a physical examination and blood and urine collection followed. Blood samples were drawn and sent overnight to the central laboratory at the University of Vermont, where they were centrifuged, aliquoted, and stored at −80°C until biomarker measurement without prior thaw.[19] The REGARDS study was approved by the institutional review boards of the participating institutions (IRB number: 201856), and all participants provided verbal consent before the telephone interview was conducted and written informed consent before completion of the in-home study visit. A single follow-up visit with the same procedures as described above for the baseline visit was conducted approximately 10 years after the baseline visit. Details of the study design have been previously described.[20]

The analytic cohort included participants selected to a nested cohort study within REGARDS, the Biomarker Mediators of Racial Disparities in Risk Factors (BioMedioR).[21]

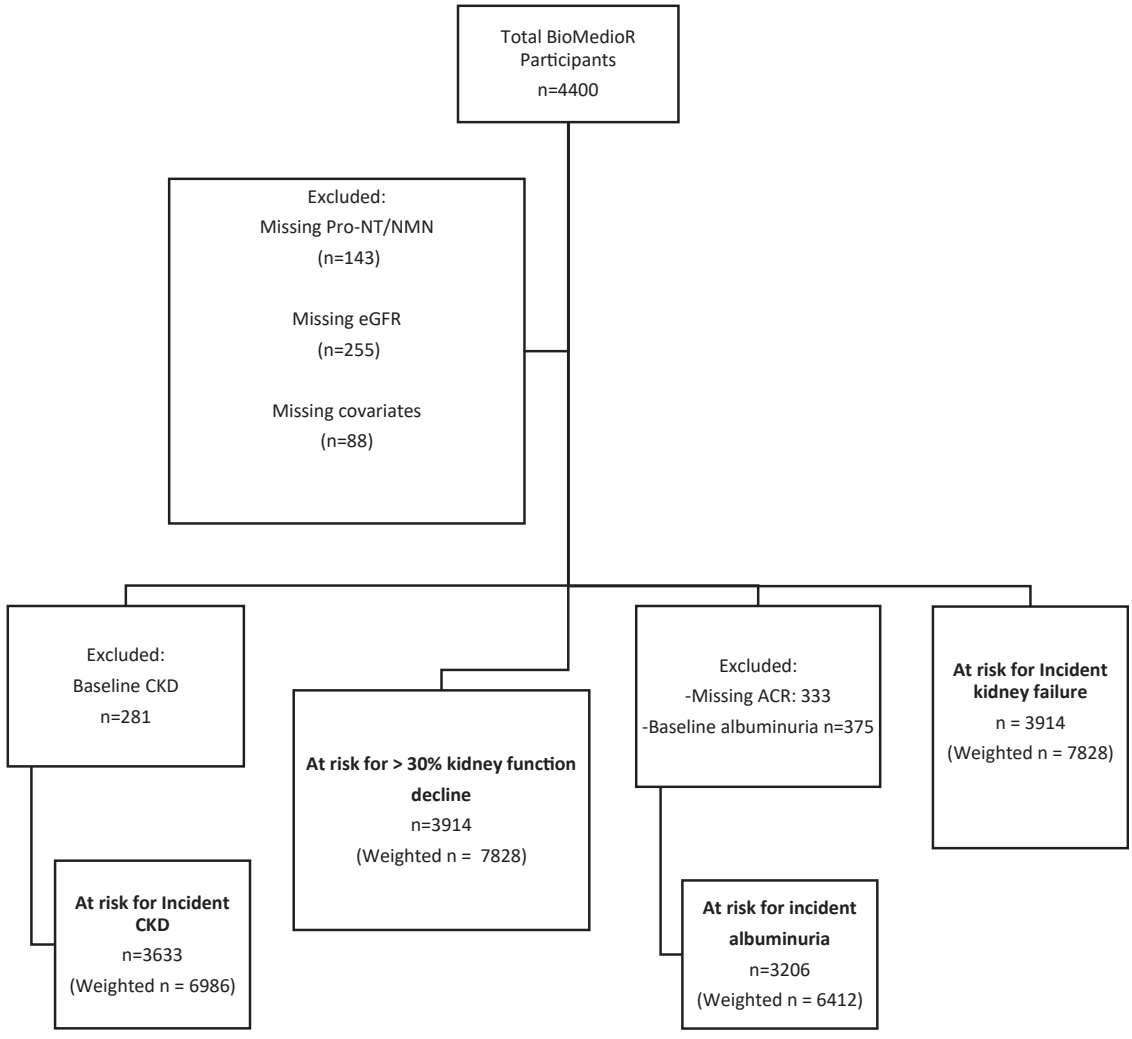

Figure 1. Flowchart of REGARDS participants for analysis. Abbreviations: ACR, albumin-creatinine ratio; BioMedioR, Biomarker Mediators of Racial Disparities in Risk Factors; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; pro-NT/NMN, proneurotensin/neuromedin N; REGARDS, REasons for Geographic And Racial Differences in Stroke.

Table 1. Baseline Characteristics of BioMedioR Participants by Pro-NT/NMN Quartile, Weighted

Pro-NT/NMN Quartiles	Q1	Q2	Q3	Q4
Range (pmol/L)	<127	127-182	183-249	250[a]
Weighted N	1,958	1,957	1,957	1,956
Age, y, mean (SD)	65 (9)	65 (8)	64 (8)	64 (8)
Women, n (%)	904 (46.1)	951 (48.6)	954 (48.9)	1010 (51.6)
Black, n (%)	702 (35.8)	872 (44.6)	1138 (58.3)	1344 (68.7)
Income, n (%)				
<$20,000	253 (12.9)	272 (13.9)	318 (16.3)	484 (24.7)
$20,000-$34,000	419 (21.4)	509 (26.0)	494 (25.2)	479 (24.5)
$35,000-$74,000	649 (33.1)	604 (30.9)	591 (30.2)	589 (30.1)
≥$75,000	390 (19.9)	348 (17.8)	334 (17.1)	264 (13.5)
Refused	249 (12.7)	224 (11.4)	220 (11.2)	140 (7.2)
Body mass index, mean (SD)	29 (6)	29 (6)	31 (6)	31 (6)
Tobacco use, n (%)	231 (11.8)	205 (10.5)	224 (11.5)	196 (10)
Cardiovascular disease, n (%)	269 (13.7)	233 (11.9)	344 (17.6)	460 (23.5)
Diabetes mellitus, n (%)	325 (16.6)	450 (23)	550 (28.2)	953 (48.7)
Metabolic syndrome, n (%)	791 (40.4)	724 (37.0)	824 (42.1)	960 (49.1)
Baseline mean systolic blood pressure, mm Hg (SD)	128 (17)	128 (16)	130 (17)	132 (17)
Baseline mean diastolic blood pressure, mm Hg (SD)	77 (8)	77 (9)	77 (10)	77 (10)
Baseline median serum creatinine, mg/dL (IQR)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)
Baseline median serum cystatin C, mg/dL (IQR)	0.9 (0.8, 1.0)	0.9 (0.8, 1.1)	1.0 (0.8, 1.1)	1 (0.9, 1.2)
Baseline median eGFR, (IQR)	92 (80, 101)	89 (78, 100)	86 (69, 99)	82 (64, 95)
Baseline median UACR, mg/g (IQR)	7 (4, 14)	8 (5, 17)	10 (4, 23)	10 (5, 33)

Abbreviations: BioMedioR, Biomarker Mediators of Racial Disparities in Risk Factors; eGFR, estimated glomerular filtration rate; IQR, interquartile range; pro-NT/NMN, proneurotensin/neuromedin N; SD, standard deviation; UACR, urinary albumin-creatinine ratio.
[a]Weighted to parent cohort and excluding participants missing pro-NT/NMN.

BioMedioR was designed to study the role of biomarkers as mediators of racial differences in incident stroke risk factors including hypertension and diabetes. This nested cohort included 4,400 individuals who completed the second visit and was deliberately sampled to obtain equal groups based on race and sex. For this study, 143 participants were excluded because they did not have pro-NT/NMN measures at baseline, 255 participants because they did not have eGFR at both the baseline and second visit, and 88 participants due to missing covariates. The final analysis included 3,914 participants (Fig 1).

According to REGARDS policy, the aims and analysis plan for this manuscript were prespecified and reviewed and approved by the REGARDS publications committee, which also reviewed the final manuscript and assured that the a priori plans were followed.

Exposure Variable

Pro-NT/NMN was measured in ethylenediaminetetraacetic acid plasma samples at an independent facility (ASKA Biotech GmbH) using a 1-step sandwich immunoluminometric sphingotest assay (SphingoTec GmbH), as previously described.[6] Laboratory personnel were blinded to clinical and demographic information. The mean coefficient of variation was 3.7%.

Covariates

Data on covariates of interest were collected at baseline. Information on age, race, sex, smoking status, lipid-lowering medication use, antihypertensive medication use, and history of coronary disease was collected by self-report. Height and weight were measured by study personnel at the in-home visit, and body mass index was calculated as weight (kg)/height squared (m^2).

Hypertension was defined as either self-reported use of antihypertensive medications, a systolic blood pressure ≥140 mm Hg, or a diastolic blood pressure of ≥90 mm Hg measured during the home examination, in which systolic blood pressure and diastolic blood pressure were the average of 2 measures taken in the seated position. Diabetes was defined as a fasting glucose ≥126 mg/dL, a nonfasting glucose ≥200 mg/dL, or current use of either oral hypoglycemic pills or insulin. Serum creatinine level was calibrated to an international isotope dilution mass spectroscopic-traceable standard, measured by colorimetric reflectance spectrophotometry (Ortho Vitros Clinical Chemistry System 950IRC, Johnson & Johnson Clinical Diagnostics, www.orthochemical.com). Serum cystatin C level was measured by particle-enhanced immunonephelometry (N Latex Cystatin C Assay, Siemens AG).[22] eGFR was calculated using the 2021 CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) creatinine- and cystatin-based equation without race coefficient.[23] Albumin and creatinine levels were measured using a random spot urine specimen by nephelometry (BN ProSpec Nephelometer, Dade Behring) and Modular-P chemistry analyzer (Roche/Hitachi), respectively. Spot urinary albumin-creatinine ratio (ACR) was calculated in mg/g.

Table 2. Association of Pro-NT/NMN Quartile With Incident CKD (Defined As eGFR <60 mL/min/1.73 m^2 and ≥40% eGFR Decline)

	Quartiles of Pro-NT/NMN					
	Q1	Q2	Q3	Q4	Per SD Higher	
	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	*P*
Range of pro-NT/NMN	<120	120-167	168-231	>232		
Number of events/Number at risk (%)						
Weighted N	107/1,752 (6.1)	117/1,742 (6.7)	162/1,746 (9.3)	210/1,746 (12)	596/6,986 (8.5)	
Model 1[a]	Reference	1.11 (0.76-1.61)	1.67 (1.17-2.35)	1.90 (1.33-2.71)	1.30 (1.14-1.47)	<0.001
Model 2[b]	Reference	1.05 (0.72-1.54)	1.44 (1.00-2.07)	1.36 (0.93-1.97)	1.14 (1.00-1.30)	0.05
Model 3[c]	Reference	1.03 (0.69-1.53)	1.46 (1.00-2.11)	1.29 (0.86-1.92)	1.11 (0.96-1.28)	0.1

Note: Weighted to parent cohort and excluding participants missing pro-NT/NMN and baseline CKD.
Abbreviations: CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; OR, odds ratio; pro-NT/NMN, proneurotensin/neuromedin N; SD, standard deviation; UACR, urinary albumin-creatinine ratio.
[a]Adjusted for age, sex, race.
[b]Adjusted for Model 1 plus body mass index, current smoking, coronary artery disease, stroke, systolic blood pressure, diastolic blood pressure, diabetes mellitus, and metabolic syndrome.
[c]Adjusted for Model 2 plus baseline eGFR and UACR.

Outcomes

The outcomes were as follows: (1) incident CKD at the second visit, defined as both eGFR of <60 mL/min/1.73 m^2 and at least 40% decline in individuals with baseline eGFR of ≥60 mL/min/1.73 m^2; (2) progressive eGFR decline, defined as ≥30% decrease in eGFR between the baseline and second in-home visit; (3) incident albuminuria, defined as a new urinary ACR ≥30 mg/g between baseline and the second in-home visit among those without baseline albuminuria; and (4) incident kidney failure ascertained by United States Renal Data System linkage up to June 2018. We also performed sensitivity analyses looking at the association between pro-NT/NMN with incident CKD defined as eGFR <60 mL/min/1.73 m^2 and at least 25% decline in individuals with baseline eGFR >60 mL/min/1.73 m^2 between baseline and the second in-home visit.[24]

Statistical Analyses

Due to the sampling design of BioMedioR, we used inverse probability sampling weights to recreate the weighted distribution of the parent REGARDS cohort and account for the sampling design. Because there are only 2 discrete visits 10 years apart, we used logistic regression with sampling weights for analysis instead of time-to-event models. Given skewed distributions, we log-transformed standardized continuous concentrations of pro-NT/NMN as the exposure of interest so that the interpretation would be per standard deviation (SD) higher level of log pro-NT/NMN. We examined the distribution of demographics and risk factors for incident CKD among pro-NT/NMN quartiles. We used sequential nested models to evaluate the association between the pro-NT/NMN and the outcomes. Covariates for multivariable models were selected a priori based on biological plausibility and were obtained at baseline. Model 1 adjusted for age, sex, and race. Model 2 additionally adjusted for body mass index, systolic blood pressure, diastolic blood pressure, diabetes mellitus, smoking, and cardiovascular disease. Model 3 additionally adjusted for baseline eGFR and urinary ACR. We tested for interactions of pro-NT/NMN with race (White vs Black) and sex (men vs women) by adding multiplicative interaction terms to model 3.

For incident kidney failure, because of the low-event rate, we only additionally adjusted for body mass index and diabetes mellitus in model 2. In the final model, we added baseline eGFR and ACR.

To assess the functional form of associations for each outcome, we also evaluated pro-NT/NMN by quartiles, setting the lowest as the reference category. When associations were observed to change monotonically across quartiles, we focused our interpretation on the results of the continuous models to maximize precision.

All analyses were conducted using STATA/PC version 16.1 (StataCorp LLC) and R version 4.1.1 (https://www.R-project.org/). *P* values of <0.05 were considered statistically significant for all analyses excluding interaction terms in which a *P* value of <0.1 was considered significant.

RESULTS

Baseline characteristics

Characteristics of study participants by quartile of pro-NT/NMN are shown in Table 1. Of the 3,914 participants, the mean ± SD age was 63 ± 8 (SD) years, 51% were women, and 48% were Black. Median baseline eGFR was 90 mL/min/1.73 m^2, and median ACR was 6 mg/g. Median pro-NT/NMN concentration was slightly higher among women and Black participants. Those with higher levels were more likely to have diabetes and higher albuminuria and lower eGFR levels.

Relationship of Pro-NT/NMN With Incident CKD

For the incident CKD analyses, we excluded 10.8% participants who had CKD at baseline. During mean follow-up

Table 3. Association of Pro-NT/NMN With Incident CKD (Defined as eGFR <60 mL/min/1.73 m^2 and ≥25% eGFR Decline)

	Quartiles of Pro-NT/NMN					
	Q1	Q2	Q3	Q4	Per SD Higher	
	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	*P*
Range of pro-NT/NMN	<120	120-167	168-231	>232		
Number of events/Number at risk (%)						
Weighted N	200/1,752 (11.4)	286/1,742 (16.4)	302/1,746 (17.3)	412/1,746 (23.6)	1,200/6,986 (17.2)	
Model 1[a]	Reference	1.50 (1.14-1.98)	1.76 (1.34-2.31)	2.38 (1.81-3.13)	1.40 (1.27-1.53)	<0.001
Model 2[b]	Reference	1.48 (1.23-2.15)	1.62 (1.23-2.15)	1.94 (1.46-2.57)	1.28 (1.16-1.42)	<0.001
Model 3[c]	Reference	1.37 (1.02-1.83)	1.48 (1.11-1.99)	1.55 (1.14-2.10)	1.16 (1.04-1.30)	0.01

Note: Weighted to parent cohort and excluding participants missing pro-NT/NMN and baseline CKD.
Abbreviations: CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; OR, odds ratio; pro-NT/NMN, proneurotensin/neuromedin N; SD, standard deviation; UACR, urinary albumin-creatinine ratio.
[a]Adjusted for age, sex, and race.
[b]Adjusted for Model 1 plus body mass index, current smoking, coronary artery disease, stroke, systolic blood pressure, diastolic blood pressure, diabetes mellitus, and metabolic syndrome.
[c]Adjusted for Model 2 plus baseline eGFR and UACR.

of 9.4 years, 8.5% participants developed incident CKD (Table 2). When pro-NT/NMN was modeled continuously, there was no significant association between pro-NT/NMN and incident CKD in the final model (odds ratio [OR], 1.10; 95% confidence interval [CI], 0.96-1.27).

In sensitivity analyses, when evaluating a less restrictive eGFR decline threshold ≥25% instead of ≥40% in addition to an eGFR of <60 mL/min/1.73 m^2, baseline pro-NT/NMN was modestly associated with incident CKD (OR, 1.16; 95% CI, 1.04-1.30), as shown in Table 3.

Relationship of Pro-NT/NMN With Progressive eGFR Decline

Among all participants, eGFR decline ≥30% was seen in 21.3% of participants. Higher pro-NT/NMN was significantly associated with progressive eGFR decline across the sequence of adjusted models (OR for fully adjusted model, 1.09; 95% CI, 1.00-1.20). Similarly, in quartile analyses, the risk of decline generally increased monotonically with higher pro-NT/NMN (Table 4).

Relationship of Pro-NT/NMN With Incident Albuminuria

After excluding participants who had prevalent albuminuria, 12.5% developed incident albuminuria. Table 5 shows the association between pro-NT/NMN and incident albuminuria. In the final model, after adjusting for baseline eGFR and ACR, there was no significant association between pro-NT/NMN and incident albuminuria (OR, 1.08; 95% CI, 0.96-1.22).

Relationship of Pro-NT/NMN With Incident Kidney Failure

Table 6 shows the relationship between pro-NT/NMN and incident kidney failure. Among 3,914 participants, incident kidney failure developed in 1.3% of participants. Each SD higher pro-NT/NMN was associated with an OR of 2.15 (95% CI, 1.67-2.78) in the model adjusted for age, race, and sex (model 1). However, in the final model including baseline eGFR, this association was attenuated and no longer significant (OR, 1.10; 95% CI, 0.83-1.46).

Table 4. Association of Pro-NT/NMN Quartile With Progressive eGFR Decline (≥30% eGFR Decline from Baseline)

	Quartiles of Pro-NT/NMN					
	Q1	Q2	Q3	Q4		
	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	Per SD Higher	*P*
Range of pro-NT/NMN	<120	120-167	168-231	>232		
Number of events/Number at risk (%)						
Weighted N	333/1,958 (17)	386/1,957 (19.7)	436/1,957 (22.3)	509/1,956 (26)	1,664/7,828 (21.3)	
Model 1[a]	Reference	1.17 (0.92-1.47)	1.37 (1.09-1.73)	1.58 (1.26-1.99)	1.22 (1.12-1.32)	<0.001
Model 2[b]	Reference	1.13 (0.89-1.44)	1.26 (1.00-1.60)	1.27 (1.00-1.61)	1.11 (1.02-1.20)	<0.02
Model 3[c]	Reference	1.12 (0.88-1.43)	1.27 (1.00-1.61)	1.23 (0.96-1.57)	1.09 (1.00-1.20)	0.04

Note: Weighted to parent cohort and excluding participants missing pro-NT/NMN. *P* for interaction for sex: 0.70. *P* for interaction for race: 0.12.
Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; pro-NT/NMN, proneurotensin/neuromedin N; SD, standard deviation; UACR, urinary albumin-creatinine ratio.
[a]Adjusted for age, sex, and race.
[b]Adjusted for Model 1 plus body mass index, current smoking, coronary artery disease, stroke, systolic blood pressure, diastolic blood pressure, diabetes mellitus, and metabolic syndrome.
[c]Adjusted for Model 2 plus baseline eGFR and UACR.

Table 5. Association of Pro-NT/NMN With Incident Albuminuria

	Quartiles of Pro-NT/NMN					
	Q1	Q2	Q3	Q4	Per SD Higher	
	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	*P*
Range of pro-NT/NMN	<120	120-167	168-231	>232		
Number of events/Number at risk (%)						
Weighted N	169/1,606 (10.5)	146/1,602 (9.1)	218/1,601 (13.6)	268/1,603 (16.7)	801/6,412 (12.5)	
Model 1[a]	Reference	0.83 (0.60-1.16)	1.35 (0.99-1.83)	1.67 (1.24-2.25)	1.33 (1.19-1.48)	<0.001
Model 2[b]	Reference	0.88 (0.64-1.22)	1.23 (0.90-1.67)	1.28 (0.94-1.75)	1.20 (1.07-1.34)	0.001
Model 3[c]	Reference	0.79 (0.56-1.11)	1.07 (0.78-1.48)	1.03 (0.75-1.43)	1.08 (0.96-1.23)	0.2

Note: Weighted to parent cohort and excluding participants missing pro-NT/NMN and baseline albuminuria.
Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; pro-NT/NMN, proneurotensin/neuromedin N; SD, standard deviation; UACR, urinary albumin-creatinine ratio.
[a]Adjusted for age, sex, and race.
[b]Adjusted for Model 1 plus body mass index, current smoking, coronary artery disease, systolic blood pressure, diastolic blood pressure, diabetes mellitus, and metabolic syndrome.
[c]Adjusted for Model 2 plus baseline eGFR and UACR.

The interactions by sex and race were not significant across the different endpoints (all *P*-interaction ≥ 0.1).

DISCUSSION

To our knowledge, this is the first study of community-living individuals to evaluate an association between plasma pro-NT/NMN and kidney function decline, albuminuria, and kidney failure. We showed that higher concentrations of pro-NT/NMN were associated with 9% greater odds of progressive eGFR decline, independent of other risk factors. However, using a strict definition of incident CKD, we did not find a significant association between pro-NT/NMN and new CKD.

It has long been recognized that diabetes mellitus, cardiovascular disease, and obesity are key risk factors in the development of CKD.[25-27] All 3 are associated with higher levels of pro-NT/NMN.[8-11,28] The precise role of pro-NT/NMN in the kidney is not yet completely understood. In murine models of CKD, an increase in circulating levels of pro-NT/NMN has been noted with very low urinary pro-NT levels, which clearly point to reduced kidney excretion.[29] However, our hypothesis is that the association between pro-NT/NMN and incident CKD goes above and beyond decreased excretion. Studies have shown the influence of neurotensinergic peptides and their receptors on inflammation and metabolism. Pro-NT reflects visceral adipose tissue inflammation, which, in turn, leads to oxidative stress and activation of the renin-angiotensin-aldosterone system.[13,30,31] Additionally, NT controls the pathways of leptin, a peptide that plays a key role in the development of hypertension and cardiovascular disease.[32] Higher levels of NT lead to higher levels of leptin. Leptin upregulates transforming growth factor β1 levels, promoting endothelial cell proliferations and fibrosis. Leptin also has proinflammatory effects leading to increased levels of tumor necrosis factor α, interleukin 1, interleukin 2, and monocyte chemoattractant protein 1, leading to accelerated atherosclerosis, insulin resistance, and endothelial dysfunction, thus promoting the development of CKD.[33]

Our study demonstrated that pro-NT/NMN relates to greater odds of eGFR decline. Associations were in similar

Table 6. Association of Pro-NT/NMN With Incident Kidney Failure

	Quartiles of Pro-NT/NMN					
	Q1	Q2	Q3	Q4	Per SD Higher	
	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	(OR, 95% CI)	*P*
Range of pro-NT/NMN	<121	121-168	169-232	>232		
Number of events/Number at risk (%)						
Weighted N	8/1,957 (0.4)	6/2,022 (0.3)	30/1,906 (1.6)	54/1,943 (2.8)	98/7,828 (1.3)	
Model 1[a]	Reference	0.68 (0.15-3.10)	3.17 (1.07-9.39)	4.64 (1.66-13.0)	2.15 (1.67-2.78)	<0.001
Model 2[b]	Reference	0.62 (0.14-2.80)	2.72 (0.90-8.24)	3.22 (1.14-9.08)	1.86 (1.44-2.41)	<0.001
Model 3[c]	Reference	0.40 (0.08-2.03)	1.76 (0.50-6.20)	0.93 (0.28-3.12)	1.10 (0.83-1.46)	0.5

Note: Weighted to parent cohort and excluding participants missing pro-NT/NMN.
Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; pro-NT/NMN, proneurotensin/neuromedin N; SD, standard deviation; UACR, urinary albumin-creatinine ratio.
[a]Adjusted for age, sex, and race.
[b]Adjusted for Model 1 plus body mass index, diabetes mellitus, and metabolic syndrome.
[c]Adjusted for Model 2 plus baseline eGFR and UACR.

directions for the other outcomes but were attenuated and not statistically significant in the final models. For kidney failure, we were limited because of the small number of events. In the incident CKD analysis, our sensitivity analysis suggested an association with a less restrictive definition of the outcome than used in our primary analyses.

In a recently published study that evaluated multiple European cohorts, the authors concluded that pro-NT/NMN was associated with impaired kidney function.[29] However, this finding was derived from a cross-sectional study in relatively homogenous cohorts. The existing data describing an association of pro-NT and impaired kidney function are limited to cross-sectional analysis of European cohorts with primarily White participants. Our study adds to this literature by providing consistent findings among a biracial of cohort of Black and White individuals in the United States. In contrast, our study sought to not only evaluate the association between pro-NT/NMN and key kidney outcomes longitudinally but also to evaluate this biomarker among Black individuals because of the unique risk factors for CKD progression relative to White persons.[34,35] There are many unanswered questions about why Black patients have a 2- to 4-fold greater risk of developing end-stage kidney disease, which is more common in Black individuals.[36,37] Part of the excess risk of CKD among Blacks can be explained by sociodemographic, lifestyle, and clinical factors such as higher rates of diabetes, hypertension, and albuminuria, yet much of the risk remains unexplained, even after accounting for genetic factors, such as apolipoprotein L-1–mediated kidney disease.[38,39] Furthermore, classic "risk factors" such as hypertension do not behave similarly in Black versus White populations with regard to CKD progression. The REGARDS study population is uniquely positioned to investigate not only whether a novel marker is a risk factor for CKD but also whether that risk differs by racial identity.

Strengths of our study include the evaluation of a cohort of White and Black men and women from regions with a high prevalence of diabetes mellitus and hypertension. This contrasts to prior studies with more homogenous cohorts.[29] We used the new creatinine–cystatin C eGFR equation without a race coefficient and a strict definition of incident CKD. A wide array of traditional CKD risk factors was robustly measured at baseline to allow evaluation of confounding.

This study has several limitations. Although REGARDS improves on the racial homogeneity of previous cohorts, results from this study are not necessarily generalizable to race groups other than non-Hispanic Whites or Blacks. We only measured pro-NT/NMN at baseline, so we were not able to evaluate longitudinal changes. eGFR and albuminuria values were only collected at 2 timepoints, ~9 years apart, so we are unable to assess shorter-term changes, and unable to address questions of informative dropout from the study because of death or illness. However, BioMedioR design assured nearly complete data on kidney disease at the 2 timepoints, allowing us to evaluate the outcomes of interest. Additionally, prior studies have not shown an impact of informative missingness in other studies in REGARDS.[40,41] It is possible that the association between pro-NT/NMN with eGFR decline could be the result of residual confounding. Also, the definition of diabetes mellitus was based on self-reported use of hypoglycemic drugs or insulin, fasting glucose or elevated random glucose, and not on A1c, which may not be sensitive enough. However, this is the diabetes definition used in REGARDS. Lastly, we had a relatively small number of kidney failure events, so confirmation of our findings is needed.

In conclusion, our data from a large, prospectively followed biracial cohort demonstrate an association between higher fasting pro-NT/NMN and progressive eGFR decline. Future studies are needed to confirm these results in other cohorts and evaluate if pro-NT/NMN may assist in the comprehensive assessment of the development of kidney disease.

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