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Fibrosis-Related Biomarkers and Incident Cardiovascular Disease in Older Adults: The Cardiovascular Health Study

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Abstract

Background—Fibrotic changes in the heart and arteries have been implicated in a diverse range of cardiovascular diseases (CVD), but whether circulating biomarkers that reflect fibrosis are associated with CVD is unknown.

Methods and Results—We determined the associations of two biomarkers of fibrosis, transforming growth factor- β (TGF-β) and procollagen type III N-terminal propeptide (PIIINP),

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with incident heart failure, myocardial infarction (MI), and stroke among community-living older adults in the Cardiovascular Health Study. We measured circulating TGF-β (n=1,371) and PIIINP (n=2,568) from plasma samples collected in 1996 and ascertained events through 2010. Given TGF-β's pleiotropic effects on inflammation and fibrogenesis, we investigated potential effect modification by C-reactive protein (CRP) in secondary analyses. After adjustment for sociodemographic, clinical, and biochemical risk factors, PIIINP was associated with total CVD (hazard ratio [HR] per standard deviation [SD]=1.07, 95% confidence interval [CI]: 1.01-1.14) and heart failure (HR per SD=1.08, CI: 1.01-1.16), but not MI or stroke. TGF-β was not associated with any CVD outcomes in the full cohort, but was associated with total CVD (HR per SD=1.16, CI: 1.02-1.31), heart failure (HR per SD=1.16, CI: 1.01-1.34), and stroke (HR per SD=1.20, CI: 1.01-1.42) among individuals with CRP above the median, 2.3 mg/L (*P*-interaction < 0.05).

Conclusions—Our findings provide large-scale, prospective evidence that circulating biomarkers of fibrosis, measured in community-living individuals late in life, are associated with CVD. Further research on whether TGF-β has a stronger fibrogenic effect in the setting of inflammation is warranted.

Keywords

collagen; cardiovascular disease; heart failure; epidemiology

Left ventricular[1] and vascular[2] remodeling are dynamic structural alterations that occur in response to growth factors, vasoactive mediators, and hemodynamic stimuli. Both types of remodeling are strong risk factors for cardiovascular disease (CVD).[1, 2] Transforming growth factor-β (TGF-β) is a matrix cytokine that modulates key features of remodeling, including cell growth, proliferation, collagen biosynthesis, and the release of collagen byproducts such as procollagen type III N-terminal propeptide (PIIINP). Circulating levels of PIIINP are strongly associated with collagen type III content in cardiovascular vessels and the myocardium.[3, 4]

TGF-β and PIIINP are associated with several CVD risk factors, including age, obesity,[5] and hypertension.[6] In prospective studies, TGF-β and PIIINP have been inconsistently associated with incident CVD events.[5, 7–12] Most of these studies have been conducted among hospitalized patients or patients with pre-existing CVD. A few studies have been conducted among community-living individuals,[9, 11, 13] but sample sizes have been modest, with the largest to date examining 922 individuals over a mean of 9.9 years.[9] Larger studies among community-living individuals of both TGF-β and PIIINP on the incidence of CVD are clearly needed. In addition, although TGF-β has dual roles as a pro-fibrotic and anti-inflammatory molecule, the interrelationships of fibrosis-related biomarkers with inflammation and CVD have yet to be clarified.[14] We evaluated the prospective associations of TGF-β and PIIINP with incident heart failure, myocardial infarction (MI), and stroke among community-living elderly individuals enrolled in the Cardiovascular Health Study.

Methods

Study Design

The design, rationale and examination details of CHS have been published elsewhere.[15] Briefly, 5,201 participants were recruited to CHS from Medicare eligibility lists in Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania in 1989–1990, and a supplemental cohort of 687 African-American participants was added in 1992–1993. To meet eligibility criteria, individuals had to be at least 65 years old, living in the community, expected to remain in the current community for at least three years after baseline, not under active cancer treatment, and able to provide written informed consent. Follow-up interviews were conducted at annual visits through 1998–1999 and at interim 6-month telephone calls, which are still ongoing. Our analysis included follow-up through 2010. All participants in our study provided written informed consent, and the institutional review board at each center approved the study protocol.

Exposure Assessment

TGF-β and PIIINP were measured in 2011–2012 from stored EDTA plasma samples from the 1996–1997 CHS visit, which is the baseline for these analyses. TFG-β was measured by ELISA (Quantikine Human TGF-β1 Immunoassay; R&D Systems, Minneapolis, MN). PIIINP was measured by the UniQ Intact N terminal Propeptide of Type III Procollagen radioimmunoassay kit manufactured by Orion Diagnostics (Fountain Hills, AZ). Inter- and intra-assay coefficients of variation (CVs) were between 1.9–2.9% and 6.4–9.3%, respectively for TFG-β. For PIIINP, inter- and intra-assay CVs were both less than 7.2%.

Because platelet contamination in plasma samples can artificially elevate levels of TGF-β,[16] we conducted pilot studies that identified probable platelet contamination at two of our four clinic sites. Hence, we measured TGF-β only at the two remaining sites, *a priori*. Characteristics of participants between sites that were included and excluded did not differ substantially, including PIIINP measurements (Supplemental Table 1). Our final analysis included 1,371 individuals free from heart failure, MI and stroke with measured levels of TGF-β, 2,568 individuals with measured levels of PIIINP, and 1,330 individuals with measured levels of both TGF-β and PIIINP.

Outcome Assessment

Cardiovascular events, including heart failure, MI, and stroke were centrally adjudicated by a CHS outcome-assessment committee on the basis of patient reports, physician diagnoses, medical records, and medication use, as previously described.[17, 18] Left ventricular ejection fraction (LVEF) was estimated from echocardiograms for patients with heart failure collected at the time of the incident event.[19] Heart failure with reduced ejection fraction (HFREF) was defined as heart failure with LVEF < 55% and heart failure with normal ejection fraction (HFNEF) was defined as a heart failure with LVEF ≥ 55%.[20]

Covariate assessment

When possible, we used covariate measurement from the 1996–1997 visit. If necessary, we carried measurements forward from the most recent visit prior to 1996–1997 with available data. We relied on self-reported age, sex, race, smoking history, leisure time physical activity (kilocalories/week), and alcohol intake (drinks/week). Use of oral hypoglycemic agents, insulin, anti-hypertensive medications (including diuretics, β-blockers, ACE inhibitors, calcium channel blockers, and other anti-hypertensive medications), or statins was verified using a validated medication inventory.[21] We imputed 685 missing values of pack years based upon age, sex, and smoking status. Trained study personnel measured systolic blood pressure (SBP). Height and weight were measured to calculate body mass index (BMI, the weight in kilograms divided by the square of the height in meters). We obtained blood and urine samples for measurement of fasting glucose, 2-hour glucose tolerance test, total cholesterol, C-reactive protein (CRP), N-terminal type B pro-brain natriuretic peptide (NT-proBNP), high sensitivity troponin-T (hsTnT), urine albumin/creatinine ratio (ACR), and estimated glomerular filtration rate (eGFR) based on measured levels of cystatin-C. We defined diabetes as fasting glucose ≥ 126 mg/dL and/or use of insulin or oral hypoglycemic medications.

Statistical analysis

We examined covariate distributions by quintile of TGF-β and PIIINP and evaluated Spearman correlation coefficients. We used Cox proportional hazards models to examine associations of TGF-β and PIIINP with heart failure, MI, and stroke, and a combined endpoint of first CVD event, using follow-up time since the 1996–1997 visit as the time scale. We evaluated hazard ratios for TGF-β and PIIINP as continuous variables (per standard deviation [SD]) and across quintiles of biomarkers. To evaluate joint associations of the biomarkers with outcomes, we created a combined variable of TGF-β and PIIINP, which was the sum of the standardized (z-score) measurements for each biomarker.

Depending on the outcome of interest, individuals with prevalent disease at baseline were excluded. Model 1 adjusted for age (strata), sex, race, and clinic site. Model 2 additionally adjusted for smoking status (current, former, never), pack years of smoking, BMI, SBP, leisure time physical activity (quintiles), alcohol use, CRP (log), total cholesterol (quintiles), diabetes, anti-hypertensive medications, statins, and prevalent forms of other CVD (heart failure, MI, and/or stroke, depending on the outcome) at baseline. We found no violations of the proportional hazards assumption in fully-adjusted models, using an interaction term for exposure and follow-up time. In sensitivity analyses, we additionally adjusted for variables that could potentially be affected by fibrosis and/or variables that were not available in the full cohort, including eGFR, urine ACR, NT-proBNP, hsTnT, and 2-hour glucose tolerance test. These markers, including urine ACR[22] and NT-proBNP,[23] are strong predictors of all-cause and cardiovascular mortality in older adults, and may be either upstream or downstream of fibrogenic pathways. We adjusted for these variables to determine whether fibrosis-related biomarkers were independently associated with CVD risk.

We assessed multiplicative interaction between TGF-β and PIIINP, and by sex, race, diabetes status, and CRP. Because we detected a significant interaction with CRP, we conducted stratified analyses that dichotomized CRP at its median value (2.3 mg/L).

For individuals with incident heart failure, we determined separate associations with HFREF, HFNEF, and unclassified heart failure, and used Lunn & McNeil competing risks models to formally compare associations across competing outcomes.[24]

All analyses were conducted in SAS (SAS Institute, Cary, North Carolina). *P*-values < 0.05 were considered statistically significant for all analyses, including interaction terms.

Results

Participant characteristics

Demographic, clinical, and laboratory characteristics of participants according to quintiles of TGF-β and PIIINP are shown in Table 1. Individuals with higher levels of either TGF-β or PIIINP were more likely to be black, less physically active, and have higher CRP. Individuals with higher levels of either biomarker were also more likely to have prevalent diabetes.[25–27] NT-proBNP levels were higher among individuals with higher PIIINP, but not among individuals with higher TGF-β. TGF-β levels were weakly positively correlated with PIIINP levels (Spearman r = 0.08, *P* = 0.001).

Risk of CVD

In age, sex, race, and clinic-adjusted models, PIIINP was associated with risk of total CVD, heart failure, and stroke (Table 2), as was the combined measure of TGF-β and PIIINP. Most associations remained statistically significant in fully-adjusted models; however the associations between PIIINP and stroke and between the combined measure and heart failure were attenuated. When tested across extreme quintiles, the highest quintile of PIIINP were associated with approximately 30% higher risk of total CVD relative to the lowest (hazard ratio [HR] = 1.34, 95% confidence interval [CI]: 1.09, 1.65). TGF-β was not associated with risk of incident CVD overall, nor did we observe an interaction between levels of TGF-β and PIIINP on risk (*P* - interaction=0.10).

In sensitivity analyses conducted among the 60% of individuals without missing values of eGFR, urine ACR, NT-proBNP, hsTnT, and 2-hour glucose tolerance test, additional adjustment for these variables did not substantially change regression coefficients.

Stratified analyses

We did not observe significant effect modification by sex, race, or diabetes status (*P* - interactions all > 0.05). However, CRP modified the associations of TGF-β with total CVD and heart failure (*P* - interactions both < 0.05). Associations of TGF-β, PIIINP, and the combined measure of TGF-β and PIIINP with total CVD, heart failure, and stroke were generally statistically significant among individuals with higher CRP (> 2.3 mg/L), and consistently larger in magnitude for individuals with higher CRP than those with lower CRP (Table 3). CRP level was modestly but positively correlated with both TGF-β (Spearman r=0.08, *P*=0.002) and PIIINP (Spearman r=0.09, *P* < 0.001).

Associations with heart failure subtypes

Using a competing risks model in the full cohort, we did not detect significant differences in the associations of either biomarker across categories of heart failure (*P*=0.27 for TGF-β, *P*=0.15 for PIIINP). There were also no clear differences across categories of heart failure among individuals with higher CRP (*P*=0.58 for TGF-β, *P*=0.49 for PIIINP).

Discussion

In this prospective, community-based study of older adults, circulating levels of fibrosis-related biomarkers were associated with multiple adverse cardiovascular outcomes. Associations for TGF-β with total CVD, heart failure, and stroke were statistically significant among individuals with higher CRP, with a similar but non-significant pattern observed for PIIINP. Our findings provide further evidence for the hypothesis that fibrosis is an important contributor to CVD among older adults, and in the case of TGF-β, particularly when combined with systemic inflammation.

We observed a significant association between PIIINP and heart failure in the entire cohort, and PIIINP levels were higher among individuals with higher levels of NT-proBNP, a marker of LV diastolic strain. Although chronic kidney disease is prevalent in older adults and an important contributor to circulating levels of PIIINP, observed associations between PIIINP and CVD were similar after adjustment for renal function (eGFR and urine ACR). In previous studies, PIIINP has been associated with multiple indicators of cardiac structure,[28] including interventricular septum thickness and left atrial diameter,[11] as well as measures of cardiac dysfunction, including E/A ratio[29] and peak E wave velocity.[11] PIIINP has also previously been associated with the risk of incident heart failure in community-living individuals[9] and older adults.[11] Our study confirms these findings, and extends them to a larger cohort of community-based individuals. Because we observed a much larger number of number of incident cardiovascular events compared to previous studies, were able to report separate associations for heart failure, myocardial infarction, and stroke, which revealed notable differences between cardiovascular endpoints. Additionally, we defined cardiovascular disease according to validated, objective clinical endpoints (heart failure, myocardial infarction, stroke), while previous studies[9] included subjective endpoints, such as angina, and transient ischemic attack, which could bias their results.

We observed an association between TGF-β and heart failure, but only among individuals with higher levels of CRP. In a previous nested case-control study of approximately 200 individuals in CHS, TGF-β was associated with risk of heart failure without stratification by CRP.[8] There are several possible explanations for our inability to detect an overall association compared to the previous study. First, the mean age at which TGF-β was measured in our study was 78 years, compared to 75 years among controls in the previous study. It is possible that the balance of TGF-β's pro-fibrotic and anti-inflammatory effects[30] changes over time, with greater opportunity for the latter effect to suppress leukocyte recruitment and prevent the formation of unstable leukocyte-rich atherosclerotic lesions later in life.[10, 12, 26, 31] Second, our study relies on an updated assay for TGF-β, which is more reproducible than the assay used in the previous study. Third, participants in our study had a median follow-up of 7.6 years before the occurrence of incident heart failure, whereas

participants in the previous study had an average follow-up of 5.4 years; a single measurement of TGF-β may be insufficient to capture the true association between fibrosis and heart failure over the longer period of follow-up in our study. Finally, although we did not detect a statistically significant association between TGF-β and heart failure, the confidence intervals of our estimates and those of the previous study are likely to overlap. As such, we cannot exclude the possibility of an association of TGF-β with heart failure in the present analysis. Notably, several studies outside of CHS have also failed to demonstrate an association between TGF-β and adverse cardiovascular outcomes.[10, 12, 26, 31] In one prospective study of 155 patients on hemodialysis, a 1 ng/mL reduction in TGF-β concentration was actually associated with a 9% increase in the risk of cardiovascular events.[12]

The ultimate association of TGF-β and CVD remains complicated because of TGF-β's pleiotropic activity. In the cardiovascular system, inflammatory and/or injurious stimuli such as shear stress, oxidized cholesterol, hemodynamic overload, and CRP[32] stimulate lymphocytes, monocytes, and other inflammatory cells to secrete TGF-β.[33] While transient TGF-β signaling is part of the normal wound healing response, sustained TGF-β signaling can lead to excess deposition of connective tissue elements, progressive remodeling, and scar formation. Because of TGF-β's multifunctional roles in both normal wound healing and pathological fibrosis, TGF-β is unlikely to be a potential therapeutic target for cardiovascular disease. However, targeting downstream regulators of fibrosis, such as the Smad receptors, may allow selective diminution of TGF-β's pro-fibrotic effects.[34]

We found significant associations of fibrosis-related biomarkers and incident stroke, but not incident MI, even in minimally adjusted models. The absence of an association between PIIINP and MI in our study contrasts with a nested case-control study conducted earlier in the course of CHS, which used a different PIIINP assay and shorter follow-up period.[13] Among identified risk factors that are common between stroke and MI, those that are more likely to lead to stroke than to MI may be disproportionately related to fibrosis.[35] Specifically, fibrosis has been implicated in both hypertension[36] and cardioembolism/atrial fibrillation,[37] two particularly strong risk factors for stroke.[38–40] Mechanistically, TGF-β increases the collagen content of arteries, resulting in arterial stiffening, reduced compliance, and increased blood pressure.[41] TGF-β may also contribute to fibrotic remodeling of the atria, though associations of TGF-β with atrial fibrillation have been mixed.[30, 42, 43] Furthermore, higher circulating levels of PIIINP have been associated with a lower probability of maintaining sinus rhythm among patients with paroxysmal atrial fibrillation.[44] The differential association of fibrosis-related biomarkers with these risk factors for stroke versus MI may explain, in part, why TGF-β and PIIINP were associated with stroke but not MI in this study.

The associations we observed in this study were consistently greatest in magnitude, and generally statistically significant, among individuals with higher levels of CRP. Emerging evidence points to a potential role for CRP in fibrosis and fibrosis-related organ damage. CRP has been shown to directly stimulate TGF-β and induce other genes that contribute to collagen deposition.[45] CRP also reflects a number of activated pathways in systemic inflammation.[46, 47] TGF-β acts via several different types of receptors, which have different

effects on extracellular matrix deposition, and its predominant signaling pathway may depend on the inflammatory milieu.[31] Although the biological plausibility of an interaction between fibrosis and inflammation is attractive, our finding requires confirmation in other cohorts.

Our investigation is novel in several respects. We conducted our study in a large, well-characterized, community-based population and an average follow-up time of more than 10 years. We were able to minimize the possibility of confounding by measuring a diverse range of covariates and including these covariates in our multivariable-adjusted models. Additionally, whereas previous studies of fibrosis have limited their analysis to markers of collagen homeostasis (e.g. matrix metalloproteinases, their tissue inhibitors, and byproducts of collagen turnover), or other isolated biomarkers, we included two complementary biomarkers of fibrosis, TGF-β and PIIINP. The combination of TGF-β and PIIINP into a combined measure further allowed us to explore putative associations.

Our study has several important limitations. First, we had limited statistical power to detect associations of TGF-β with CVD due to the limited number of TGF-β measurements. Second, plasma levels of both biomarkers were measured at a single point and it is possible that the longitudinal trajectory of change in TGF-β or PIIINP may provide additional information, independent of the baseline level, on the future risk of CVD. Third, associations in this study may be biased towards the null because of measurement error resulting from the long hiatus between sample collection in 1996–1997 and biomarker measurement in 2005. Fourth, neither TGF-β nor PIIINP is specific to cardiac fibrosis and thus associations with CVD outcomes may be partly shadowed by fibrotic processes in other organs.

Although both TGF-β and PIIINP have previously been used as markers of tissue fibrosis in epidemiologic studies,[48] they are imperfect measures of underlying tissue fibrosis. The gold standard for evaluation of myocardial or vascular fibrosis is tissue biopsy, but even then fibrosis can be missed if it is not homogeneously distributed.[49] Several imaging modalities can also detect organ fibrosis with high sensitivity and specificity, but their use in longitudinal human studies has not been widespread.[28] Plasma biomarkers such as TGF-β and PIIINP provide a readily-available and non-invasive assessment of fibrosis appropriate for cohort studies. However, given the relatively modest observed hazard ratios for TGF-β and PIIINP and their measurement issues, neither biomarker is likely to be immediately useful for prognosis in clinical care. Nonetheless, because these markers only imperfectly reflect underlying fibrosis, the potential benefits of targeting fibrosis may be larger than the observed hazard ratios for TGF-β and PIIINP might suggest. Given that several anti-fibrotic agents are already in development or testing,[46, 50] clinical trials to specifically target fibrosis and determine its effects on cardiovascular disease such as heart failure and stroke may be feasible in the near future.

In conclusion, PIIINP and, in the setting of high CRP, TGF-β are associated with some forms of CVD among older adults. Our findings provide support for future research on fibrosis as a potentially targetable pathway to reduce cardiovascular morbidity and mortality. In the setting of clinical trials, TGF-β and PIIINP could potentially be used to

identify target populations for interventions and/or to monitor response to therapy. Further research on whether TGF-β has a stronger adverse effect on cardiovascular disease in the setting of increased inflammation is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of Cardiovascular Health Study Participants by Quintile of Each Biomarker, 1996–1997

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Among 1,627 individuals with measured levels of TGF- β					
TGF-β (ng/L)	1,489 (267)	2,288 (279)	3,482 (401)	5,447 (830)	10,927 (4,498)
PIIINP (ng/mL)	4.7 (1.6)	4.9 (1.8)	4.9 (1.6)	5.0 (1.8)	5.2 (2.0)
Age (years)	77 (4)	78 (5)	78 (5)	78 (4)	78 (5)
Male (%)	39	41	41	45	37
Black Race (%)	20	17	19	20	31
Former Smoking (%)	37	52	50	49	44
Current Smoking (%)	12	5	7	9	13
BMI (kg/m^2)	27 (5)	27 (5)	27 (5)	27 (4)	27 (5)
SBP (mmHg)	134 (19)	136 (21)	133 (20)	133 (21)	136 (21)
Physical activity (kilocalories/week)*	780 (1,353)	767 (1,358)	698 (1,335)	549 (1,373)	613 (1,122)
Alcohol use (drinks/week)*	0.0 (0.5)	0.0 (0.5)	0.0 (1.0)	0.0 (1.0)	0.0 (0.5)
CRP (mg/L)*	2.2 (3.8)	2.4 (4.1)	2.4 (3.5)	2.2 (4.3)	3.3 (6.3)
Total cholesterol (mg/dL)	195 (38)	202 (39)	198 (37)	200 (41)	203 (41)
Diabetes (%)	14	14	15	17	20
Anti-hypertensive medications (%)	47	55	58	57	62
Statins (%)	12	10	11	10	10
eGFR (mL/min/1.73m^2)	74 (16)	72 (22)	70 (20)	71 (20)	70 (19)
Urine ACR (mg/g)*	9 (10)	10 (16)	9 (14)	10 (16)	9 (17)
NT-proBNP (pg/mL)*	107 (144)	119 (186)	122 (185)	103 (167)	105 (144)
Among 3,237 individuals with measured levels of PIIINP					
TGF-β (ng/L)	4,312 (3,549)	4,862 (4,726)	4,693 (3,820)	4,820 (3,900)	5,001 (3,695)
PIIINP (ng/mL)	3.0 (0.4)	3.9 (0.2)	4.4 (0.2)	5.3 (0.3)	7.5 (2.0)
Age (years)	77 (4)	78 (5)	78 (5)	78 (5)	79 (5)
Male (%)	33	39	39	45	45
Black Race (%)	11	14	14	16	22
Former Smoking (%)	42	43	45	49	46

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Current Smoking (%)	10	8	7	7	7
BMI (kg/m^2)	26 (4)	27 (4)	27 (5)	27 (5)	28 (5)
SBP (mmHg)	137 (21)	137 (21)	136 (20)	137 (21)	137 (21)
Physical activity (kilocalories/week)*	863 (1,428)	814 (1,459)	698 (1,466)	660 (1,423)	540 (1,155)
Alcohol use (drinks/week)*	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	0.0 (0.5)
CRP (mg/L)*	2.2 (3.8)	2.3 (3.9)	2.3 (4.1)	2.5 (4.2)	2.7 (4.2)
Total cholesterol (mg/dL)	206 (38)	204 (38)	204 (40)	198 (39)	199 (42)
Diabetes (%)	13	12	14	16	21
Anti-hypertensive medications (%)	54	55	55	61	65
Statins	10	9	9	11	10
eGFR (mL/min/1.73m^2)	77 (18)	74 (18)	71 (19)	67 (19)	63 (19)
Urine ACR (mg/g)*	10 (14)	9 (13)	9 (14)	11 (23)	12 (30)
NT-proBNP (pg/mL)	116 (143)	118 (148)	116 (166)	136 (189)	138 (220)

Abbreviations - Body mass index (BMI), Confidence interval (CI), C-reactive protein (CRP), Estimated glomerular filtration rate (eGFR), N-terminal type B pro-brain natriuretic peptide (NT-proBNP), Procollagen type III N-terminal propeptide (PIIINP), Systolic blood pressure (SBP), Transforming growth factor-β (TGF-β), Urine albumin/creatinine ratio (ACR)

Except where indicated, continuous variables are reported as mean (standard deviation).

Dichotomous and categorical variables are reported as %.

*Reported as median (interquartile range).

Table 2

Multivariable-adjusted hazard ratios (per standard deviation) for incident cardiovascular disease (CVD) among Cardiovascular Health Study participants

	TGF-β				PIIINP				Combined			
	N (cases/total)	**HR**	**95% CI**	***P***	**N (cases/total)**	**HR**	**95% CI**	***P***	**N (cases/total)**	**HR**	**95% CI**	***P***
Total CVD												
Model 1*	540/1371	1.08	0.99, 1.19	0.08	**1017/2568**	**1.11**	**1.05, 1.18**	**<0.001**	**525/1330**	**1.15**	**1.06, 1.26**	**0.002**
Model 2†	540/1371	1.06	0.97, 1.17	0.19	**1017/2568**	**1.07**	**1.01, 1.14**	**0.03**	**525/1330**	**1.12**	**1.02, 1.23**	**0.02**
Heart Failure												
Model 1*	432/1555	1.06	0.96, 1.17	0.28	**855/2940**	**1.13**	**1.06, 1.21**	**<0.001**	**422/1577**	**1.11**	**1.00, 1.23**	**0.04**
Model 2†	432/1555	1.05	0.94, 1.17	0.39	**855/2940**	**1.08**	**1.01, 1.16**	**0.03**	422/1577	1.07	0.96, 1.19	0.20
Myocardial infarction												
Model 1*	225/1510	1.04	0.90, 1.20	0.57	452/2882	1.05	0.95, 1.16	0.32	216/1465	1.00	0.87, 1.17	0.91
Model 2†	225/1510	1.04	0.90, 1.20	0.61	452/2882	1.03	0.93, 1.14	0.60	216/1465	0.99	0.85, 1.15	0.86
Stroke												
Model 1*	249/1600	1.13	0.99, 1.28	0.07	**454/3036**	**1.10**	**1.01, 1.20**	**0.04**	**241/1549**	**1.22**	**1.08, 1.39**	**0.002**
Model 2†	249/1600	1.12	0.98, 1.28	0.10	454/3036	1.07	0.98, 1.17	0.16	**241/1549**	**1.22**	**1.08, 1.39**	**0.002**

Abbreviations – Cardiovascular disease (CVD), Confidence interval (CI), Hazard ratio (HR), Procollagen type III N-terminal propeptide (PIIINP), Transforming growth factor-β (TGF-β)

1 Standard deviation = 3951.75 ng/L (TGF-β); 1.78 ng/mL (PIIINP)

*Adjusted for age, sex, race, clinic.

†Adjusted for age, sex, race, clinic, smoking status, pack years, body mass index, systolic blood pressure, physical activity, alcohol use, C-reactive protein, total cholesterol, diabetes, hypertension medications, statins.

Table 3

Multivariable-adjusted hazard ratios (per standard deviation) for incident cardiovascular disease (CVD) among Cardiovascular Health Study participants, stratified by C-reactive protein (CRP)

	TGF-β					PIIINP					Combined				
	N (cases/total)	**HR**	**95% CI**	***P***	***P*–int**	**N (cases/total)**	**HR**	**95% CI**	***P***	***P*–int**	**N (cases/total)**	**HR**	**95% CI**	***P***	***P*–int**
Total CVD					**0.05**					0.29					0.24
High CRP	**285/673**	**1.16**	**1.02, 1.31**	**0.02**		**535/1248**	**1.14**	**1.04, 1.25**	**0.006**		**278/648**	**1.21**	**1.06, 1.38**	**0.005**	
Low CRP	255/698	0.96	0.82, 1.13	0.60		482/1320	1.02	0.92, 1.12	0.71		247/682	1.04	0.90, 1.20	0.60	
Heart Failure					**0.05**					0.61					0.30
High CRP	**233/776**	**1.16**	**1.01, 1.34**	**0.04**		467/1457	1.10	1.00, 1.21	0.05		228/749	1.14	0.99, 1.32	0.08	
Low CRP	199/779	0.93	0.77, 1.12	0.43		388/1483	1.05	0.94, 1.17	0.37		194/758	1.01	0.85, 1.19	0.94	
Myocardial infarction					0.59					0.89					0.71
High CRP	124/760	1.09	0.90, 1.33	0.38		260/1433	1.08	0.94, 1.24	0.27		122/734	1.05	0.85, 1.29	0.67	
Low CRP	101/750	1.02	0.81, 1.30	0.84		192/1449	0.97	0.82, 1.14	0.68		94/731	0.98	0.77, 1.24	0.87	
Stroke					0.39					0.24					0.12
High CRP	**140/806**	**1.20**	**1.01, 1.42**	**0.04**		**244/1515**	**1.14**	**1.00, 1.29**	**0.05**		**135/777**	**1.36**	**1.15, 1.61**	**< 0.001**	
Low CRP	109/794	1.04	0.82, 1.33	0.73		210/1521	1.03	0. 90, 1.18	0.69		106/772	1.10	0.88, 1.37	0.43	

Abbreviations –Cardiovascular disease (CVD), Confidence interval (CI), C-reactive protein (CRP), Hazard ratio (HR), Procollagen type III N-terminal propeptide (PIIINP), Transforming growth factor-β (TGF-β)

1 Standard deviation = 3951.75 ng/L (TGF-β); 1.78 ng/mL (PIIINP)

High vs. Low CRP is dichotomized at CRP's median value, 2.3 mg/dL.

P-interactions were calculated using CRP as a continuous variable.

Adjusted for age, sex, race, clinic, smoking status, pack years, body mass index, systolic blood pressure, physical activity, alcohol use, CRP, total cholesterol, diabetes, hypertension medications, statins, prevalent cardiovascular disease (year 9).