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Circulating C-Type Natriuretic Peptide and its Relationship to Cardiovascular Disease in the General Population

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Abstract

C-type natriuretic peptide (CNP) is an endothelium-derived peptide that is released as a protective mechanism in response cardiovascular injury and/or disease. However no studies have investigated circulating CNP, identifying clinical factors that may influence CNP and its relationship to cardiovascular disease in the general population. We studied 1,841 randomly selected subjects from Olmsted County, Minnesota, USA (mean age 63 ± 11 years; 48% male). Plasma CNP was measured by a well-established radioimmunoassay and echocardiography, clinical characterization and detailed medical record review were performed. We report that CNP circulates at various concentrations (median 13 pg/ml), was unaffected by gender, was weakly associated by age and that highest quartile of CNP identified a high-risk phenotype. Subjects with CNP in the highest quartile were associated with increased risk of myocardial infarction (multi-variable adjusted hazard ratio, 1.51; 95% confidence interval, 1.09–2.09; $P = 0.01$) but not heart failure, cerebrovascular accidents or death over a follow-up of 12 years. Addition of the highest quartile of CNP to clinical variables led to a modest increase in the integrated discrimination improvement for risk of myocardial infarction. In a large community-based cohort elevated circulating CNP identified a high-risk phenotype that included cardiovascular comorbidities and left ventricular dysfunction, and provided evidence that highest concentrations of CNP potentially has prognostic value in predicting future risk of myocardial infarction. Together, these data from the general population highlights the potential value of CNP and supports the need for additional studies to evaluate whether mechanisms regulating CNP could improve outcomes.

Keywords

C-Type Natriuretic Peptide; Myocardial Infarction; Biological Markers; Endothelium; Cardiovascular Diseases

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DISCLOSURES

Drs. Sangaralingham and Burnett and Ms. Heublein are named as coinventors on patent application relating to the use of CNP as a biomarker.

INTRODUCTION

C-type natriuretic peptide (CNP) is a family member of the cardiovascular (CV) hormones that include atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). Unlike ANP and BNP which are primarily produced in cardiomyocytes,^{1, 2} CNP is an endothelial product^{3, 4} synthesized in a number of vascular beds including the heart and is released in response to stimuli including shear stress and cytokines.⁴⁻⁶ Experimental studies have demonstrated the ability of CNP to suppress cardiac fibroblast proliferation, collagen synthesis and myocardial fibrosis⁷⁻¹⁰ inhibit vascular smooth muscle proliferation and migration,¹¹⁻¹³ induce coronary vasodilation,¹⁴ regulate vascular homeostasis,^{15, 16} promote cardiomyocyte relaxation,¹⁷ and stimulate endothelial cell regeneration.¹⁸ Importantly, human investigations have reported that CNP is secreted from the heart,^{19, 20} head and neck²⁰ of patients with CV disease, is significantly elevated in advanced atherosclerotic lesions^{21, 22} and in the circulation of heart failure (HF) patients²³ and has prognostic value in HF patients with preserved ejection fraction.²⁴ Thus, these beneficial actions observed in experimental studies point to numerous potential mechanisms by which CNP may be protective from CV disease progression, while human studies may be indicative of underlying CV injury and/or disease.

As evidence continues to mount for the biological significance of CNP and potential protective activation in CV injury and/or disease, no studies to date have assessed circulating CNP and its relationship to CV disease in the general population. Such a study would provide insights into pathophysiology of CNP from human population perspective, as well as may identify sub-clinical CV injury and/or disease in a group of subjects, who in turn, may be at risk for future adverse CV events. Therefore we conducted for the first time, a comprehensive study to define and identify factors that may influence plasma CNP; and to evaluate the potential prognostic value of CNP for adverse CV outcomes using a well characterized and randomly selected community- based cohort. We hypothesized that: 1) plasma CNP circulates at various concentrations; 2) is modulated by age and gender; and 3) would be associated with CV dysfunction and have prognostic significance for adverse CV outcomes.

METHODS

Methods are provided in the online-only Data Supplement.

RESULTS

Baseline Study Sample Characterization

Baseline characteristics by quartiles of plasma CNP of the 1,841 subjects are shown in Table 1. The mean age of the 1,841 subjects was 63 ± 11 years and 48% were male. Subjects in the highest quartile of plasma CNP were older, were more likely to have documented coronary artery disease (CAD), prior myocardial infarction (MI) and atrial fibrillation/flutter, were more likely to be on CV medication and to have elevated systolic blood pressure (BP) as well as left atrial volume index (LAVI), regional wall motion abnormality (RWMA), mitral

peak velocity of early filling (E) and mitral peak velocity of early filling to medial mitral annular tissue velocity (E/e') ratio.

The distribution of plasma CNP by age and gender is shown in Figure S1 with regression lines by gender. Here, there was a weak positive association with plasma CNP and age ($r=0.06$; $P=0.01$) and no evidence of gender differences. Furthermore, plasma CNP concentrations ranged from 2 pg/ml to 265 pg/ml, with median (Q1, Q3) of 13.2 (10.2, 16.8) pg/ml. Moreover, correlations between plasma CNP and BNP ($r=0.14$, $P<0.001$) and NT-proBNP ($r=0.11$, $P<0.001$) were low in magnitude and there was no evidence of a correlation with plasma CNP and eGFR ($r=-0.01$; $P=0.54$). In age and gender adjusted univariate analysis (Table 2), older age, higher prevalence of CAD, prior MI, CV medication use, RWMA and elevated ventricular filling pressures (E and E/e') as well as higher plasma levels of proBNP, BNP, NT-proBNP and C-reactive protein (CRP) were significantly associated with plasma CNP being in the highest quartile. Adjusted means of plasma CNP by quartiles of proBNP, BNP and NT-proBNP is shown in Figure S2. While there is a general trend upwards in CNP levels with increasing BNP levels, particularly proBNP and BNP, the most striking association is seen in the highest quartile. In the age and gender adjusted multivariable analysis (Table 3), higher BNP and CRP were independent predictors of plasma CNP in the highest quartile.

Cardiovascular Morbidity and Mortality

During a median follow-up of 12.1 years, there were 189 MIs, 232 HF events, 350 cerebrovascular accidents (CVAs) and 328 all-cause death, among the 1,841 subjects. Figure 1 shows Kaplan-Meier curves for the unadjusted cumulative incidence of MI, HF, CVAs and death in all subjects according quartiles of CNP. MI, HF and mortality risk significantly increased among subjects with CNP in the highest quartile relative to those subjects in the lower three quartiles of CNP. No such trend occurred for risk of CVAs. Age-, gender- and BMI-adjusted as well as multivariable adjusted hazard ratios for MI, HF, CVA and death according to baseline CNP in the highest quartile (versus the lower three quartiles), as well as a continuous variable, are presented in Table 4. Plasma CNP in the highest quartile was independently associated with significant increased risk for MI after adjustment for traditional CV risk factors, CRP, RWMA, E and plasma NT-proBNP. In contrast, plasma CNP was not independently associated with increased risk for HF, CVAs (Table 4 and Table S1) or mortality after adjustment for traditional CV risk factors, CRP, RWMA, E and plasma NT-proBNP.

Plasma CNP in the Prediction of Myocardial Infarction

We further evaluated the incremental predictive value of highest quartile of CNP when added to the base model of traditional CV risk factors that included age, gender, BMI, total cholesterol, serum creatinine, smoking, the presence of diabetes mellitus, hypertension and CAD as well as CRP, RWMA and E (Table S2; Model 2). The addition of highest quartile of CNP to the latter model for predicting risk of MI resulted in a similar c-statistic of 0.82 (0.79–0.85) and also led to a modest improvement in the IDI ($\pm$ SE) [0.005 ± 0.003 ; $P=0.05$] and a relative IDI of 10.4%.

DISCUSSION

In this first community based study, we found that CNP circulates at various concentrations, was unaffected by gender, was weakly associated by age and that highest concentrations of CNP identified a high-risk phenotype that included CV comorbidities and LV dysfunction. Furthermore, CNP in the highest quartile was significantly prognostic for risk of MI after adjustment for the model that included traditional CV risk factors, CRP, RWMA and E and plasma NT-proBNP and added modest incremental predictive value for risk of MI. Together these findings from our general population cohort highlights the pathophysiological relevance and biomarker potential of plasma CNP in CV disease.

Historically CNP is thought to circulate at low concentrations in adults,²⁵ that suggest that CNP functions as an autocrine/paracrine factor. Here we report that plasma CNP levels in adult subjects range from 2 to 265 pg/ml, thus providing evidence that CNP may function beyond a local factor. Our data also demonstrated that the phenotype of subjects with highest CNP were older, had elevated systolic BP, had an increased prevalence of CAD, prior MI, atrial fibrillation/flutter, HF and use of CV medication, thus supporting a cross-sectional association between elevated CNP and CV comorbidities. The elevations in circulating CNP levels, and its independent associations with high BNP and CRP, in these subjects may be reflective of the pathophysiological effects and pathogenesis of underlying CV injury, disease and/or an inflammatory state as seen with atherosclerosis. Indeed, highest quartile of proBNP and BNP and its association to higher CNP is suggestive that each captures a distinct aspect of pathophysiology, where CNP may be more related to vascular disease and the BNP molecular forms maybe more related myocardial disease. Nevertheless, the increase in plasma CNP observed our general population study is likely multifactorial and exact mechanism(s) for its elevation remains to be fully elucidated. First, it is tempting to speculate that CNP activation may serve as both a protective response to adverse CV remodeling and systemic inflammation, as well as a potential marker of injury recognizing that hypoxia²⁶ due to ischemia secondary to underlying CAD and pro-inflammatory/-fibrotic cytokines and growth factors^{4, 6} are known stimuli for CNP production and release. Secondly, the elevated CNP levels may also be a reflection of a compensatory mechanism to underlying myocardial fibrogenesis as experimental evidence has demonstrated that elevations in CNP prevent cardiac fibrosis in vitro⁷ and in vivo¹⁰ and a deficiency in CNP may result in cardiac fibrosis and dysfunction.^{8, 9} Our echocardiography data found that myocardial regional wall motion abnormalities, higher left atrial volume index, impaired diastolic function and elevated ventricular filling pressure (E and E/e') were associated with elevated plasma CNP. The changes in these echocardiographic parameters may reflect underlying myocardial fibrosis where hemodynamic and/or biochemical stimuli may activate CNP production and release from the fibroblast and endothelial cell to suppress the profibrotic process.²⁰ Nevertheless, the contributing factors and alterations that trigger CNP activation, release, and result in elevated circulating CNP, maybe a reflection of spillover into the systemic circulation. Lastly, another potential explanation is that the subjects who have elevated CNP levels may have a greater frequency of the recently reported CNP gene (NPPC) single-nucleotide polymorphisms (rs11079028 or rs4796751), which result in greater circulating levels.²⁷ At this point given the complex nature of CNP, additional

studies are needed to investigate the potential mechanisms by which CNP is produced and released in both the coronary circulation, vasculature and the myocardium in high-risk subjects identified by elevated CNP.

It is also of interest that CNP was unaffected by gender and was weakly associated by age. This finding related to age and gender is unique to CNP, as ANP and BNP have been reported to have a meaningful increase with age and are higher in females.^{28–31} The association, albeit weak, of age and CNP in our study is consistent with finding by Prickett *et al.*³² However elevation in natriuretic peptide (NP) levels, particularly BNP, seen in older adults and in females in these previous human studies has been in part attributed to the influence of sex hormones. This proposed relationship has been supported by experimental studies, which have demonstrated that androgens are suppressive, while estrogen stimulates the NPs.^{33, 34} Our data would suggest that the regulation of CNP production from the endothelium is distinct from the other NPs being release from the cardiomyocytes and may not be affected by sex hormones. However, further studies are needed to confirm this speculation.

Myocardial infarction is the major CV consequence of CAD and the development of post-MI HF remains a leading cause of morbidity and mortality. Thus, this trend underscores the importance of primary prevention³⁵ and the increasing interest in the identification of potential new biomarkers for risk prediction in the general population. Interestingly, our multivariable models demonstrated that the highest concentration of CNP was prognostic for MI, but not for HF, CVAs or mortality even after adjusting for traditional CV risk factors, CRP, RWMA, E and plasma NT-proBNP. Importantly, the phenotype of the subjects with the highest risk of MI as identified with highest CNP provides confidence that CNP is associated with CAD and its sequelae, MI. Notably, the data suggests that the highest quartile of CNP may add some incremental predictive value to established CV risk factors, CRP, RWMA and E in identifying subjects at future risk for MI. Together, these findings have potential biomarker implications given the prognostic value of elevated plasma CNP to identify subjects in the general community at increased risk for future MI. Thus, continued evaluation of the clinical utility of plasma CNP in other population cohorts with and without CV disease warrants further investigations.

A strength of our study is that our cohort is a large, well-characterized, random sample of the general population. Further, the median follow-up of 12 years duration and documentation of all-cause mortality and CV events are also a notable strengths. However there are also some limitations that include a predominantly Caucasian population and only adults of the age 45 years old. Hence, our results may not be generalizable to other ethnicities and age groups. We also report all-cause mortality because of the determination of specific causes of death were too few for analyses. Additionally, events in our study were based on ICD-9 codes and the non-fatal events have not been adjudicated.

PERSPECTIVES

Our study is the first to explore the potential role of CNP in the development of CV dysfunction and disease from a human perspective using a well-characterized, random

sample of the general population. Our findings characterize the phenotype of subjects with elevated plasma CNP and are suggestive that highest concentrations of CNP may have prognostic value in predicting future risk of MI in these subjects. Our results support the need for additional studies in other and more diverse populations to continue to evaluate the biomarker potential of CNP in identifying subject at risk for CV disease progression and development of adverse CV events such as MI. Future studies are also warranted to explore the potential mechanisms linking CNP production to CV abnormalities and whether implementing therapeutic strategies, CNP-based or others, prevent disease progression and improve outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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NOVELTY AND SIGNIFICANCE

1) What is New?

1. We demonstrate that in a general population cohort, CNP circulates beyond low concentrations, was unaffected by gender, was weakly associated by age and that highest concentrations of CNP identified a high-risk phenotype.
2. Plasma CNP in the highest quartile was significantly prognostic for risk of MI after adjustment for traditional CV risk factors, C-reactive protein (CRP), regional wall motion abnormalities (RWMAs), mitral peak velocity of early filling (E) and plasma NT-proBNP.
3. Plasma CNP in the highest quartile added modest incremental predictive value to traditional CV risk factors, CRP, RWMA and E for risk of MI in this cohort.

2) What is Relevant?

- The results of this study characterizes the phenotype of subjects with elevated plasma CNP and suggest that highest concentrations of CNP may have modest incremental prognostic value in predicting future risk of MI in the general community.
- This general population investigation broadens our understanding of the pathophysiological relevance of CNP and the potential significance of this peptide as a biomarker that is suggestive of a compensatory protective mechanism to underlying CV injury and/or disease.

Summary

Further translational studies are needed in other and more diverse populations to confirm the potential role of CNP in the development of CV dysfunction and disease, and to evaluate the mechanistic link of CNP production and release to CV abnormalities. The results of such future studies may be useful in testing the efficacy of therapeutic strategies, CNP-based or others, to prevent disease progression and improve outcomes.

Figure 1.
Kaplein-Meier curves for unadjusted cumulative incidence of myocardial infarction (A), heart failure (B), cerebrovascular accidents (C) and mortality (D) according to quartiles of plasma CNP.

Table 1

Baseline Characteristics According to Quartiles of Plasma CNP Level.

Variable	Plasma CNP by Quartile, pg/ml				P Value
	2.0–10.1 (n=450)	10.2–13.1 (n=455)	13.2–16.7 (n=459)	16.8 (n=477)	
Age at Exam, yr	62 (10)	63 (11)	63 (11)	64 (11)	0.001
Gender					0.70
. Male	219 (49%)	224 (49%)	216 (47%)	218 (46%)	
. Female	231 (51%)	231 (51%)	243 (53%)	259 (54%)	
BMI, kg/m ²	28 (5)	29 (5)	28 (5)	29 (6)	0.46
Hypertension	125 (28%)	119 (26%)	122 (27%)	151 (32%)	0.22
Diabetes	28 (6%)	39 (9%)	29 (6%)	39 (8%)	0.39
CAD	45 (10%)	51 (11%)	52 (11%)	77 (16%)	0.021
Prior MI	19 (4%)	17 (4%)	18 (4%)	38 (8%)	0.007
Past HF Diagnosis	9 (2%)	4 (1%)	11 (2%)	17 (4%)	0.049
Cardiomyopathy	8 (2%)	6 (1%)	9 (2%)	9 (2%)	0.88
COPD	14 (3%)	20 (4%)	23 (5%)	23 (5%)	0.49
Smoking Status					0.22
. Never	216 (48%)	211 (46%)	240 (52%)	249 (53%)	
. Former	192 (43%)	206 (45%)	172 (38%)	188 (40%)	
. Current	41 (9%)	38 (8%)	46 (10%)	36 (8%)	
Valve Disease	18 (4%)	11 (2%)	23 (5%)	25 (5%)	0.13
Atrial Fib/Flutter	14 (3%)	18 (4%)	23 (5%)	36 (8%)	0.011
CV Drug	152 (34%)	159 (35%)	154 (34%)	199 (42%)	0.027
ACE Inhibitor	38 (9%)	31 (7%)	37 (9%)	50 (11%)	0.28
Beta Blocker	61 (15%)	60 (14%)	67 (16%)	82 (18%)	0.35
CCB	23 (6%)	36 (9%)	28 (7%)	43 (10%)	0.10
Diuretic	72 (17%)	73 (18%)	68 (16%)	98 (22%)	0.12
Anti-lipidemic	68 (16%)	82 (20%)	68 (16%)	88 (20%)	0.33
Systolic BP, mmHg	132 (22)	134 (20)	132 (22)	135 (23)	0.035
Diastolic BP, mmHg	74 (11)	74 (10)	73 (10)	74 (10)	0.11
Total Cholesterol, mg/dl	201 (178, 226)	201 (182, 227)	203 (180, 225)	199 (177, 222)	0.33

Variable	Plasma CNP by Quartile, pg/ml					P Value
	2.0–10.1 (n=450)	10.2–13.1 (n=455)	13.2–16.7 (n=459)	16.8 (n=477)		
Serum Creatinine, mg/dl	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)		0.69
eGFR, ml/min/1.73 m ²	82 (72, 89)	83 (73, 90)	82 (70, 90)	81 (68, 89)		0.21
eGFR 60 ml/min/1.73 m ²	396 (88%)	399 (88%)	382 (83%)	403 (84%)		0.10
NT-proBNP, pg/ml	59.8 (27.5, 125.8)	66.7 (25.7, 123.8)	73.5 (26.6, 163.4)	82.2 (35.2, 211.0)		<0.001
proBNP, pg/ml	16.0 (7.0, 35.0)	18.0 (9.0, 36.0)	22.5 (11.0, 48.5)	23.5 (10.0, 53.0)		<0.001
BNP (Biosite), pg/ml	18.7 (8.8, 41.3)	22.5 (8.7, 44.0)	27.7 (10.5, 59.9)	30.9 (12.4, 74.5)		<0.001
BNP (Biosite, 56.1 pg/ml)*	77 (18%)	78 (18%)	122 (28%)	160 (35%)		<0.001
CNP, pg/ml	8.4 (7.0, 9.4)	11.6 (10.8, 12.4)	14.6 (13.9, 15.6)	20.2 (18.3, 23.1)		<0.001
CRP, ug/ml	3.0 (1.0, 14.0)	3.5 (1.0, 14.0)	3.8 (1.0, 14.0)	5.3 (1.4, 14.0)		0.002
CRP (> 14 ug/ml)*	142 (32%)	144 (32%)	152 (33%)	205 (43%)		<0.001
Left Atrial Enlargement	59 (14%)	63 (15%)	79 (19%)	85 (19%)		0.12
LV Hypertrophy	37 (11%)	45 (13%)	42 (12%)	58 (15%)		0.22
EF, %	63 (7)	63 (7)	63 (7)	62 (8)		0.06
EF Category, %						0.20
• 50	432 (96%)	439 (96%)	441 (96%)	447 (94%)		
• 40–49	15 (3%)	11 (2%)	13 (3%)	18 (4%)		
• < 40	3 (1%)	5 (1%)	5 (1%)	12 (3%)		
LAVI, ml/m ²	24.1 (7.9)	24.2 (7.5)	25.8 (15.9)	26.1 (9.5)		0.008
RWMA	13 (3%)	17 (4%)	23 (5%)	38 (8%)		0.002
E, m/sec	0.66 (0.17)	0.65 (0.15)	0.68 (0.17)	0.69 (0.16)		0.006
E/e' ratio	8.39 (3.13)	8.37 (2.95)	8.75 (2.98)	9.11 (3.30)		0.002
LV Dimension Index	2.63 (0.31)	2.64 (0.33)	2.62 (0.30)	2.63 (0.31)		0.84
LV Mass Index, g/m ²	184 (50)	187 (53)	182 (49)	183 (53)		0.67
Diastolic Function						0.41
• Normal	296 (73%)	290 (69%)	279 (69%)	286 (69%)		
• Mild	81 (20%)	93 (22%)	80 (20%)	81 (19%)		
• Moderate/Severe	31 (8%)	38 (9%)	43 (11%)	50 (12%)		

Values are mean (±standard deviation), n (%) or median (interquartile range).

* Highest (top) quartile.

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BMI = body mass index; CAD = coronary artery disease; MI = myocardial infarction; HF = heart failure; COPD = chronic obstructive pulmonary disease; Fib = fibrillation; CV = cardiovascular; ACE = angiotensin-converting enzyme; CCB = calcium channel blocker; BP = blood pressure; eGFR = estimated glomerular filtration rate; NT-proBNP = amino-terminal pro-B-type natriuretic peptide; proBNP = pro-B-type natriuretic peptide; BNP = B-type natriuretic peptide; CNP = C-type natriuretic peptide; CRP = C-reactive protein; LV = left ventricular; EF = ejection fraction; LAVI = left atrial volume index; RWMA = regional wall motion abnormality; E = mitral peak velocity of early filling; E/e' = ratio of mitral peak velocity of early filling to medial mitral annular tissue velocity.

Table 2

Age and Gender Adjusted Variables That Significantly Contributes to Plasma CNP in the Highest Quartile (Q4) in Univariate Analyses

Variable	Odd Ratio	95% CI	P Value
Age	1.02	1.01–1.03	0.001
CV Disease[§]			
CAD	1.47	1.07–2.02	0.02
MI	1.93	1.24–3.01	0.004
Atrial Fib/Flutter	1.74	1.11–2.72	0.02
CV Drug[§]	1.26	1.01–1.58	0.05
Biomarkers[§]			
NT-proBNP*	1.21	1.09–1.34	0.0003
proBNP*	1.21	1.09–1.33	0.0003
BNP*	1.25	1.13–1.39	<0.0001
CRP (ordinal quartiles)	1.20	1.10–1.32	<0.0001
Echocardiography[§]			
RWMA	2.04	1.31–3.18	0.002
E	2.30	1.21–4.38	0.01
E/e'	1.06	1.02–1.10	0.007

* = transformed using natural logarithm.

[§] = Age and Gender Adjusted.

Abbreviations same as in Table 1.

Table 3

Age and Gender Adjusted Variables That Significantly Contributes to Plasma CNP in the Highest Quartile (Q4) in Multivariable Analysis

Variable	Odd Ratio	95% CI	P Value
BNP*	1.89	1.45–2.45	<0.0001
CRP*	1.58	1.26–1.98	<0.0001

* Top Quartile.

Abbreviations same as in Table 1.

Table 4

Mortality and Cardiovascular Morbidity in the General Population During 12 Years of Follow-Up According to Baseline Plasma CNP.

Outcome (No of events)	HR (95% CI) per 1 SD Increase in Log Variable	P Value	HR (95% CI) for Plasma CNP Values in the Highest Quartile (Q4)*	P Value
Myocardial Infarction (n=189)				
Unadjusted	1.60 (1.19–2.16)	0.002	1.87 (1.40–2.51)	< 0.0001
Adjusted for age, gender and BMI	1.70 (1.22–2.37)	0.002	1.80 (1.34–2.41)	< 0.0001
Multivariable model 1	1.59 (1.13–2.24)	0.008	1.67 (1.23–2.26)	0.0009
Multivariable model 2	1.51 (1.05–2.17)	0.02	1.59 (1.16–2.18)	0.004
Multivariable model 3	1.41 (0.97–2.06)	0.07	1.51 (1.09–2.09)	0.01
Heart Failure (n=232)				
Unadjusted	1.35 (1.02–1.79)	0.04	1.76 (1.35–2.30)	< 0.0001
Adjusted for age, gender and BMI	1.28 (0.94–1.74)	0.12	1.52 (1.17–1.99)	0.002
Multivariable model 1	1.25 (0.92–1.71)	0.15	1.47 (1.12–1.94)	0.006
Multivariable model 2	1.06 (0.75–1.49)	0.74	1.32 (0.99–1.77)	0.06
Multivariable model 3	0.96 (0.67–1.38)	0.83	1.26 (0.94–1.70)	0.13
Cerebrovascular Accidents (n=350)				
Unadjusted	1.22 (0.97–1.54)	0.09	1.25 (0.99–1.57)	0.06
Adjusted for age, gender and BMI	1.15 (0.90–1.47)	0.27	1.11 (0.88–1.40)	0.38
Multivariable model 1	1.18 (0.92–1.51)	0.19	1.09 (0.86–1.37)	0.49
Multivariable model 2	1.19 (0.92–1.55)	0.18	1.10 (0.86–1.40)	0.46
Multivariable model 3	1.12 (0.86–1.47)	0.41	1.06 (0.82–1.36)	0.67
Death (n=328)				
Unadjusted	1.41 (1.11–1.79)	0.004	1.41 (1.12–1.78)	0.004
Adjusted for age, gender and BMI	1.33 (1.02–1.73)	0.04	1.26 (1.00–1.58)	0.05
Multivariable model 1	1.34 (1.02–1.75)	0.04	1.26 (0.99–1.61)	0.06
Multivariable model 2	1.23 (0.92–1.64)	0.17	1.18 (0.91–1.52)	0.21
Multivariable model 3	1.07 (0.79–1.45)	0.66	1.06 (0.82–1.38)	0.66

* Hazard ratio for subjects with values in the highest quartile relative to the rest of subjects in the lower three quartiles. The highest quartile corresponds to values 16.8 pg/ml for plasma CNP.

Multivariable model 1: Adjustment for age, gender, BMI, total cholesterol, serum creatinine, smoking, the presence of diabetes mellitus, hypertension and CAD.

Multivariable model 2: Adjustment for variables in model 1 plus CRP, RWMA and E.

Multivariable model 3: Adjustment for variables in model 2 plus NT-proBNP.