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Relative B-Type Natriuretic Peptide Deficiency May Exist in Diastolic Dysfunction in Subclinical Population

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Background: Heart failure patients are deficient in B-type natriuretic peptide (BNP) but the significance of subclinical BNP deficiency is unclear.

Methods and Results: A total of 1,398 subjects without cardiovascular disease, with left ventricular ejection fraction (LVEF) ≥50% and BNP level <100 pg/mL, were selected from a 2005–2008 health checkup in Arita-cho, Japan, and divided into 2 groups: with and without LV diastolic dysfunction (DD+ or DD–). We performed propensity score matching on non-cardiac factors affecting BNP levels and analyzed 470 subjects in each group (372/940 men; median age, 66 years). The DD(+) group showed higher lateral E/e', an index of estimated left ventricular filling pressure, and greater prevalence of concentric hypertrophy (CH) despite similar BNP levels, suggesting a relative deficiency of BNP in DD(+) compared with DD(–). Multivariable logistic regression analysis revealed an increase in BNP correlated with decreased odds of CH (adjusted odds ratio [aOR] 0.663, 95% confidence interval (CI) 0.484–0.909, P=0.011), whereas an increase in lateral E/e' was associated with increased odds of CH (aOR, 2.881; 95% CI, 1.390–5.973; P=0.004). Furthermore, CH in combination with diastolic dysfunction independently predicted major adverse cardiovascular events (hazard ratio 3.272, 95% CI 1.215–8.809; P=0.019).

Conclusions: Relative BNP deficiency was associated with CH, which had a poor prognosis in patients with diastolic dysfunction.

Key Words: B-type natriuretic peptide; Diastolic dysfunction; Left ventricular hypertrophy

Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), encoded by the *NPPA* and *NPPB* genes, respectively, are released from cardiac myocytes into the circulation in response to myocardial wall stretching by elevated left ventricular filling pressure (LVFP). Thus, the plasma natriuretic peptide (NP) levels are clinically valuable biomarkers for assessing the presence and severity of cardiac hemodynamic stress.[1,2] Elevated LVFP is one of the most important factors that trigger not only LV hypertrophy (LVH) but also the transcriptional activation of NPs. A positive correlation between high plasma BNP levels, LVH, and diastolic dysfunction has been demonstrated in patients with heart failure (HF) or hypertension.[3,4] Considering the cardiovascular protective properties of NPs, such as their natriuretic, vasodilative, antihypertrophic, and antifibrotic effects, NPs are believed to be compensatorily released to relieve hemodynamic stress.

However, recently, some patients with HF reportedly did not have elevated BNP levels, even when they were hospitalized for HF or had abnormalities in cardiac structure or function.[5] Moreover, approximately 25% of patients with HF with preserved ejection fraction (EF) have normal plasma BNP levels.[6] These facts suggest an "endocrine deficiency of NPs",[5] in which the elevation of NPs is insufficient to counteract an elevated LVFP. NP deficiency

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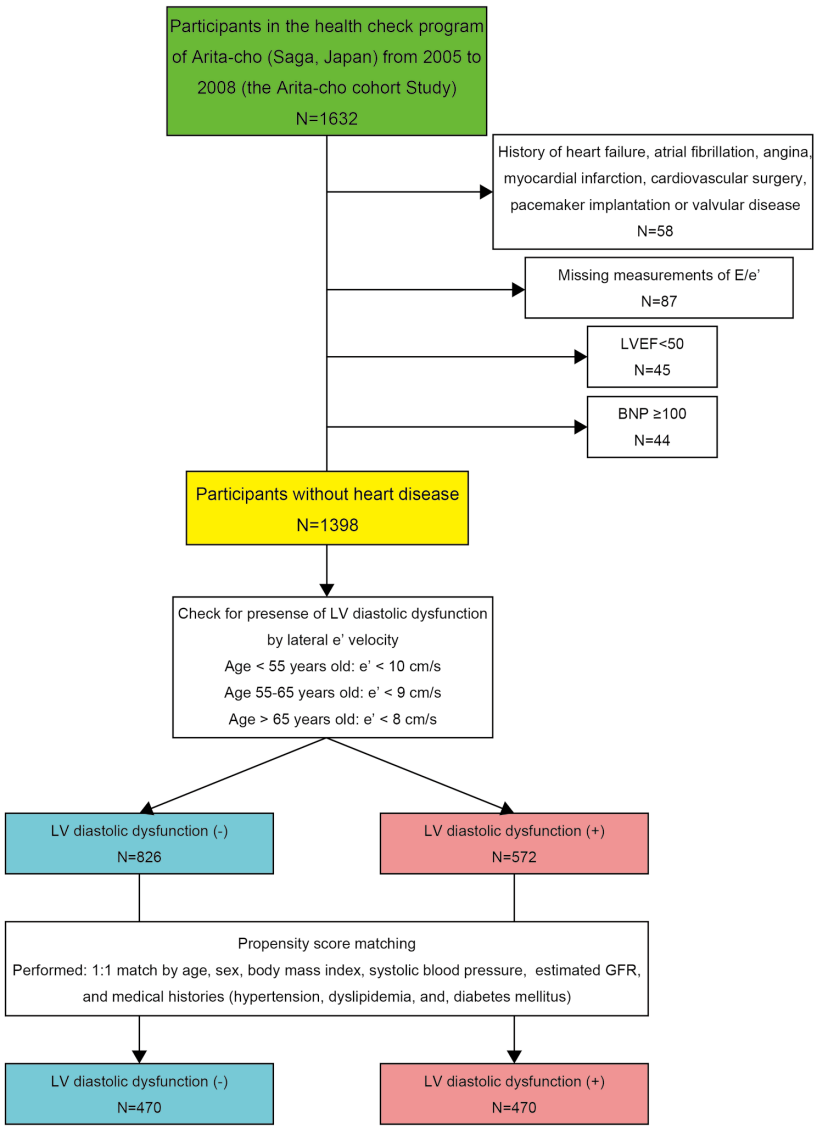

Figure 1. Study flowchart. The study population included 1,632 participants in the Arita-cho health check program from 2005 to 2008 (the Arita-cho cohort study); we enrolled 1,398 participants without heart disease and divided them into "no LV diastolic dysfunction" and "LV diastolic dysfunction" groups. We then used propensity score matching for age, sex, body mass index, systolic blood pressure, eGFR, and medical history (hypertension, dyslipidemia, and diabetes mellitus). Ultimately, we analyzed 470 participants in each group for a total of 940 participants. BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; LV, left ventricle; LVEF, left ventricular ejection fraction.

predisposed the development of LVH in a mouse model.[7] Genetic variations in or near *NPPA*, *NPPB*, and *NPRA* encoding the NP receptor A (NPRA) are also associated with increased cardiac wall thickness and LV mass (LVM) in humans.[8,9] These animal and human studies strongly support the hypothesis that NP deficiency contributes to LVH. However, no clinical studies have demonstrated an association between lower plasma BNP levels and LVH in humans, possibly because patients with HF have elevated LVFP, which upregulates BNP expression in parallel with LVH as a morphological adaptation.[10] Intriguingly, we previously demonstrated that lower plasma BNP levels were linked to LV concentric remodeling, which is defined as increased cardiac wall thickness and a preclinical stage of LVH, in participants of a health checkup program whose plasma BNP levels were within the normal physiological range (<35 pg/mL).[11] In addition, we recently reported that LV concentric hypertrophy (CH) was associated with higher estimated LVFP without a proportional increase in plasma BNP levels in individuals with plasma BNP <100 pg/mL within a subclinical population defined as population with undetected cardiovascular disease.[12] These findings suggest an inverse association between concentric changes in LV geometry and plasma BNP levels, and made us to hypothesize that a lower plasma BNP level relative to LVFP, what is called NP deficiency, is a risk for LVH, which may predispose to diastolic dysfunction, in a subclinical population. To test this hypothesis, we investigated the association between plasma BNP levels, LV geometry, LV diastolic dysfunction, and estimated LVFP, by using a clinically oriented algorithm that incorporated age, lateral e', and E/e' ratio,[13] in subclinical participants of a health checkup program with no previous history of cardiac disease.

Methods

The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committees of the National Cerebral and Cardiovascular Center and

Table 1. Clinical and Laboratory Data of Each Group With and Without LV Diastolic Dysfunction

	All cohort (N=940)	LV diastolic dysfunction (–) (N=470)	LV diastolic dysfunction (+) (N=470)	P value
Age, years	66 (61–71)	67 (61–71)	66 (61–71)	0.609
Male, n (%)	372 (39.6)	188 (40.0)	184 (39.1)	0.790
Body mass index, kg/m^2	22.6 (20.7–24.5)	22.4 (20.6–24.6)	22.8 (20.9–24.5)	0.345
Smoker (%)				0.053
Current	116 (12.3)	46 (9.8)	70 (14.9)	
Past	160 (17.0)	85 (18.1)	75 (16.0)	
None	664 (70.6)	339 (72.1)	325 (69.1)	
Alcohol intake (%)				0.756
Every day	240 (25.5)	125 (26.6)	115 (24.5)	
Sometimes	203 (21.6)	100 (21.3)	103 (21.9)	
Never	497 (52.9)	245 (52.1)	252 (53.6)	
Systolic blood pressure, mmHg	134 (122–147)	133 (122–145)	135 (123–149)	0.185
Diastolic blood pressure, mmHg	81 (74–89)	81 (73–87)	82 (75–90)	0.002
Pulse rate, beats/min	65 (59–72)	64 (59–72)	65 (59–72)	0.401
Medical history				
Hypertension, n (%)	284 (30.2)	138 (29.4)	146 (31.1)	0.570
Dyslipidemia, n (%)	118 (12.6)	60 (12.8)	58 (12.3)	0.844
Diabetes mellitus, n (%)	69 (7.3)	34 (7.2)	35 (7.4)	0.900
Laboratory data				
AST, IU/L	22.5 (20.0–27.0)	23.0 (19.0–27.0)	22.0 (20.0–28.0)	0.254
ALT, IU/L	18.0 (14.0–25.0)	18.0 (14.0–23.0)	19.0 (15.0–26.0)	0.031
γ–GTP, IU/L	24.0 (17.0–39.0)	23.0 (17.0–37.0)	24.0 (17.0–40.0)	0.194
Albumin, g/dL	4.6 (4.5–4.8)	4.6 (4.4–4.8)	4.7 (4.5–4.8)	0.016
Fasting blood glucose, mg/dL	89 (83–97)	88 (83–96)	90 (84–97)	0.083
Hemoglobin A1c, %	5.6 (5.4–5.9)	5.6 (5.4–5.8)	5.7 (5.4–5.9)	0.001
Total cholesterol, mg/dL	207 (186–229)	200 (182–225)	211 (190–232)	0.001
Triglyceride, mg/dL	95 (69–129)	90 (66–119)	99 (74–138)	<0.001
HDL-cholesterol, mg/dL	58 (49–68)	58 (50–68)	58 (49–67)	0.528
LDL-cholesterol, mg/dL	126 (107–146)	123 (106–146)	128 (109–147)	0.051
Adiponectin, μg/mL	9.9 (6.8–14.1)	10.3 (7.0–14.2)	9.4 (6.5–13.8)	0.091
eGFR, mL/min/1.73 m^2	76.7 (65.4–91.2)	76.7 (65.4–91.3)	77.0 (66.0–91.3)	0.770
Hemoglobin, g/dL	13.7 (12.8–14.6)	13.6 (12.7–14.5)	13.7 (12.9–14.7)	0.024
Platelets, 10^4/μL	22.0 (19.0–25.6)	21.5 (18.2–25.6)	22.3 (19.3–25.6)	0.342
BNP, pg/mL	21.0 (11.6–34.5)	20.0 (11.6–35.7)	21.4 (11.8–33.8)	0.604

Numeric values are expressed as mean }standard deviation or median (interquartile range). γ-GTP, γ-glutamyl transpeptidase; ALT, alanine transaminase; AST, aspartate transaminase; BNP, B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LV, left ventricular.

Arita-cho (M28-077-3, M14-025-6). Written informed consent was given by all participants before the study.

Study Population and Propensity Score Matching

The study population included 1,632 participants of the Arita-cho Health Check Program (Saga, Japan) from 2005 to 2008 (Arita-cho cohort study).[11,14] Participants with a history of cardiovascular disease such as HF, atrial fibrillation, angina, myocardial infarction, cardiovascular surgery, pacemaker implantation, and/or valvular disease at baseline were excluded, as were those who did not undergo measurements of their plasma BNP level or were missing data for parameters of Doppler echocardiography, including lateral e'. Moreover, we excluded patients with BNP ≥100 and/or LVEF <50% to avoid undetected HF.[15] We enrolled 1,398 participants from the Arita-cho cohort study and divided them into no LV diastolic dysfunction (DD−) and LV diastolic dysfunction (DD+) groups according to age and lateral e', which is less susceptible to right ventricular influences.[13] We then used propensity score matching for age, sex, body mass index, systolic blood pressure, and estimated glomerular filtration rate (eGFR), which are non-cardiac factors that can affect plasma BNP levels, and medical history (hypertension, dyslipidemia, and diabetes mellitus) based on previous clinical studies.[1,16] The matching ratio of the groups was 1:1, and logistic regression was used to calculate the propensity scores. The nearest neighbor method with a 0.2 caliper restriction was used for propensity score matching. Ultimately, we analyzed 470 subjects in each group, for a total of 940 subjects (**Figure 1**).

Laboratory Tests and Echocardiographic Analysis

Blood was collected from each participant ≥10 h after the last food intake, and the plasma BNP concentration was measured using a commercial immunoradiometric assay

Table 2. Echocardiographic Parameters of Each Group With or Without LV Diastolic Dysfunction

	All subjects (N=940)	LV diastolic dysfunction (–) (N=470)	LV diastolic dysfunction (+) (N=470)	P value
Echocardiographic parameters				
Interventricular septal thickness, mm	9.0 (8.0–10.0)	9.0 (7.9–10.0)	9.0 (8.0–10.0)	0.052
Posterior wall thickness, mm	9.0 (8.0–10.0)	9.0 (8.0–10.0)	9.0 (8.0–10.0)	0.036
LV end-diastolic dimension, mm	46.0 (43.0–49.0)	46.0 (43.0–49.0)	46.0 (43.0–49.0)	0.826
LV end-systolic dimension, mm	27.0 (24.0–30.0)	27.0 (24.0–30.0)	27.0 (24.0–31.0)	0.488
LV ejection fraction, %	65.8 (59.6–71.5)	66.0 (60.3–71.5)	65.6 (58.9–71.6)	0.511
Relative wall thickness, mm	0.39 (0.35–0.44)	0.39 (0.34–0.43)	0.39 (0.35–0.44)	0.092
LV mass, g	136 (112–164)	133 (110–159)	138 (114–164)	0.034
LV mass index, g/m^2	88 (75–104)	86 (74–101)	91 (76–106)	0.009
LA diameter, mm	37 (33–40)	37 (33–40)	37 (33–40)	0.319
E-wave velocity, m/s	60 (50–70)	64 (54–75)	55 (47–65)	<0.001
A wave velocity, m/s	73 (62–85)	75 (64–85)	72 (60–85)	0.221
E/A ratio	0.82 (0.70–0.95)	0.85 (0.73–1.00)	0.78 (0.66–0.91)	<0.001
Deceleration time, ms	213 (183–250)	217 (188–250)	213 (183–250)	0.866
Septal e', cm/s	6.0 (5.0–7.3)	6.9 (5.9–8.0)	5.3 (4.4–6.2)	<0.001
Septal a', cm/s	9.3 (8.0–10.7)	9.9 (8.5–11.0)	8.8 (7.7–10.0)	<0.001
Septal E/e'	9.9 (8.1–12.0)	9.3 (7.8–11.1)	10.6 (8.7–12.8)	<0.001
Lateral e', cm/s	8.6 (7.0–10.2)	10.2 (9.3–11.5)	7.0 (5.9–7.9)	<0.001
Lateral a', cm/s	10.0 (8.5–11.7)	10.6 (8.9–12.3)	9.8 (8.2–11.1)	<0.001
Llateral E/e'	7.1 (5.8–8.6)	6.2 (5.1–7.4)	8.3 (6.9–10.0)	<0.001
Average E/e'	8.5 (7.1–10.3)	7.7 (6.6–9.1)	9.6 (8.0–11.5)	<0.001
Estimated LV filling pressure				<0.001
Likely normal (lateral E/e' <8)	614 (65.3)	404 (86.0)	210 (44.7)	
Intermediate (lateral E/e' 8–12)	279 (29.7)	65 (13.8)	214 (45.5)	
Likely high (lateral E/e' >12)	47 (5.0)	1 (0.2)	46 (9.8)	
LV geometry				0.008
Normal	490 (52.1)	256 (54.5)	234 (49.8)	
Concentric remodeling	204 (21.7)	110 (23.4)	94 (20.0)	
Concentric hypertrophy	105 (11.2)	37 (7.9)	68 (14.5)	
Eccentric hypertrophy	141 (15.0)	67 (14.3)	74 (15.7)	

Numeric values are expressed as the mean±standard deviation or median (interquartile range). LA, left atrium; LV, left ventricular.

kit for human BNP (Shionoria; Shionogi, Osaka, Japan).

Experienced technicians performed echocardiography using commercially available ultrasonography systems. Assessments were performed in accordance with the American Society of Echocardiography (ASE).[17,18] LV end-diastolic and end-systolic diameters (LVDd and LVDs, respectively), interventricular septal thickness, LV posterior wall thickness (PWT), peak E-wave, and septal and lateral e' velocities were measured. Right wall thickness (RWT) and LVM were calculated according to the ASE guidelines, and the LVM index (LVMI) was calculated with correction for body surface area: RWT [mm] = 2PWT/LVDd, LVM [g] = $0.8\{1.04[(\text{LVDd} + \text{interventricular septal thickness} + \text{PWT})^3 - (\text{LVDd})^3]\} + 0.6$. RWT was considered to increase if >0.42, and LVMI was considered to increase if LVMI was >115/95 in men and women, which is the definition of LVH.[19] These analyses generated 4 categories: normal, concentric remodeling, eccentric hypertrophy, and CH. In patients with normal geometry, neither RWT nor LVMI increased. Concentric remodeling was defined as normal LVMI but increased RWT; eccentric hypertrophy was defined as increased LVMI with normal RWT; and CH was defined as increased LVMI and RWT. LV diastolic dysfunction and estimated LVFP were evaluated using an existing algorithm.[13] Specifically, the presence of LV diastolic dysfunction was assessed by age and lateral e' velocity and defined as follows: age <55 years, e' <10 cm/s; 55–65 years, e' <9 cm/s; and age >65 years, e' <8 cm/s. The estimated LVFP was evaluated using lateral E/e' as a continuous variable and 3 categorical variables: likely normal (lateral E/e' <8), intermediate (lateral E/e' 8–12), and likely high (lateral E/e' >12).

Clinical Events During Follow-up

Medical history was obtained using a standardized questionnaire at baseline and during the annual health check-ups. If a participant provided information suggestive of new-onset cardiovascular disease, we obtained confirmation from the clinic or hospital where the participant consulted a cardiologist. This study evaluated all-cause death and major adverse cardiovascular events (MACE: composite endpoint of cardiovascular death, the occurrence of acute myocardial infarction and stroke, hospitalization for HF, and ischemic cardiovascular events) for prognosis. For participants without clinical events, the final follow-up date was the date of last contact.

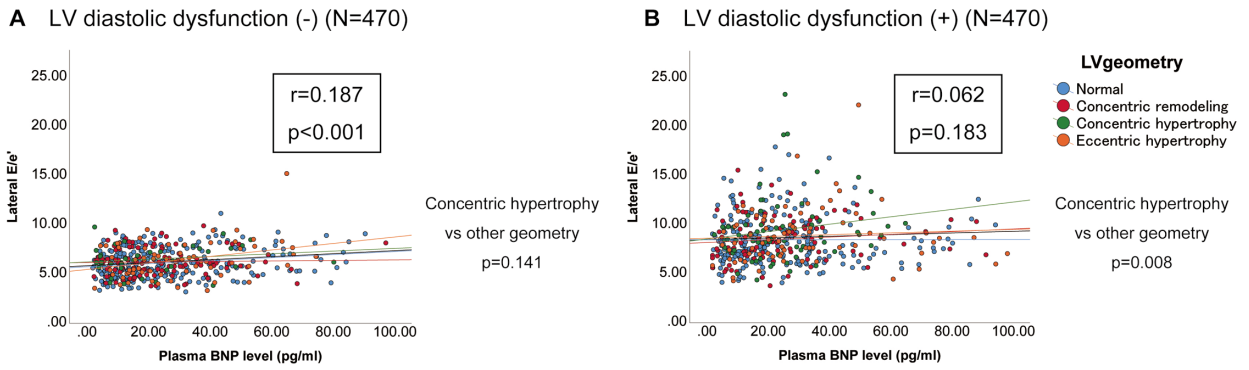

Figure 2. Correlation between lateral E/e' and plasma BNP levels in subjects with and without LV diastolic dysfunction. (**A**) Correlation in subjects without LV diastolic dysfunction (N=470), showed a significant positive correlation between lateral E/e' and plasma BNP levels (r=0.187, P<0.001). (**B**) Same correlation in subjects with LV diastolic dysfunction (N=470) was not statistically significant (r=0.062, P=0.183). Data points are color-coded based on LV geometries: normal geometry is shown in blue, concentric remodeling in red, concentric hypertrophy in green, and eccentric hypertrophy in orange. The effect of concentric hypertrophy on the correlation between lateral E/e' and plasma BNP levels is highlighted, with a significantly different pattern observed in subjects with LV diastolic dysfunction (P=0.008) compared with other geometries, in contrast to those without (P=0.141). BNP, B-type natriuretic peptide; LV, left ventricle; r, Pearson's correlation coefficient.

Statistical Analysis

Data are expressed as mean±standard deviation if the variable was normally distributed or median (interquartile range) if not. As appropriate, groups were compared using the Student's t-test or Wilcoxon test for continuous values and the chi-squared test for categorical data. The Shapiro–Wilk test was used to assess whether the data were normally distributed. All tests were two-sided, and statistical significance was set at P<0.05. Correlations were assessed using Pearson's correlation coefficient (r), and differences between groups were assessed using analysis of covariance (ANCOVA). Multiple logistic regression analysis investigated whether plasma BNP levels and lateral E/e' are associated with LV geometry. Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were calculated. As potential confounders, factors that were biologically essential and considered to be associated with the dependent variables in previous studies were included in the multivariate analyses. There were no missing data for variables included in the multivariate analysis. Multicollinearity among the variables in the model was assessed by calculating the variance inflation factor and correlation coefficient. Kaplan-Meier analysis was used to evaluate clinical events during follow-up, and differences in survival curves were tested using a log-rank (Mantel–Cox) test. Cox proportional hazards analysis was performed to evaluate the influence of CH on events. All statistical analyses were performed using SPSS software (version 26; SPSS Inc., Chicago, IL, USA).

Results

Estimated LVFP Relative to Plasma BNP Level and Prevalence of CH in DD+ Group

Among the subjects in the Arita-cho health checkup program, 1,398 subclinical individuals without heart disease or reduced LV systolic function (LVEF <50%) were divided into 2 groups using non-invasive Doppler echocardiography according to a clinically oriented algorithm of LV diastolic dysfunction and estimated LVFP: DD− (n=826) and DD+ (n=572). Thereafter, 470 individuals in each group were selected using propensity score matching for parameters that affected plasma BNP levels and medical histories. **Table 1** shows the clinical characteristics and laboratory data of the patients. There was no significant difference between groups for the items used for matching age (67 (61–71) vs. 67 (61–71) years, P=0.609), male sex (188 (40.1%) vs. 184 (39.1%), P=0.790), body mass index (22.4 (20.6–24.6) vs. 22.8 (20.9–24.5) kg/m^2, P=0.345), systolic blood pressure (133 (122–145) vs. 135 (123–149) mmHg, P=0.185), eGFR (76.7 (65.4–91.3) vs. 77.0 (66.0–91.3) mL/min, P=0.770), and prevalence of hypertension (138 (29.4%) vs. 146 (31.1%), P=0.570), dyslipidemia (60 (12.8%) vs. 58 (12.3%), P=0.844), and diabetes mellitus (34 (7.2%) vs. 35 (7.4%), P=0.900). The DD+ group had higher diastolic blood pressure (81 (73–87) vs. 82 (75–90) mmHg, P=0.002) and higher alanine transaminase (18.0 (14.0–23.0) vs. 19.0 (15.0–26.0) IU/L, P=0.031), albumin (4.6 (4.4–4.8) vs. 4.7 (4.5–4.8) g/dL, P=0.016), hemoglobin A1c (5.6 (5.4–5.8) vs. 5.7 (5.4–5.9) %, P=0.001), total cholesterol (200 (182–225) vs. 211 (190–232) mg/dL, P=0.001), triglyceride (90 (66–119) vs. 99 (74–138) mg/dL, P<0.001), and hemoglobin (13.6 (12.7–14.5) vs. 13.7 (12.9–14.7) g/dL, P=0.024) levels. Unexpectedly, plasma BNP levels did not differ between groups (DD+ 20.0 (11.6–35.7) vs. DD− 21.4 (11.8–33.8) pg/mL, P=0.604).

Next, we analyzed the echocardiographic parameters (**Table 2**). The DD+ group had lower septal and lateral e' (6.9 (5.9–8.0) vs. 5.3 (4.4–6.2) cm/s, P<0.001, 10.2 (9.3–11.5) vs. 7.0 (5.9–7.9) cm/s, P<0.001, respectively) and higher septal, lateral, and average E/e' (9.3 (7.8–11.1) vs. 10.6 (8.7–12.8), P<0.001, 6.2 (5.1–7.4) vs. 8.3 (6.9–10.0), P<0.001, 7.7 (6.6–9.1) vs. 9.6 (8.0–11.5), P<0.001, respectively) than the DD− group. Even when divided into 3 groups (likely normal/intermediate/likely high) according to the algorithm for estimating LVFP by lateral E/e',[13] intermediate and likely high LVFP were more common in the DD+ group (65 (13.8%) vs. 214 (45.5%), 1 (0.2%) vs. 46 (9.8%), P<0.001, respectively). These results suggested

Table 3. Logistic Regression Analysis Examining the BNP Levels and Lateral E/e' for Predicting Abnormal LV Geometries

Variable	LV concentric remodeling vs. normal geometry			LV concentric hypertrophy vs. normal geometry			LV eccentric hypertrophy vs. normal geometry		
	aOR	(95% CI)	P value	aOR	(95% CI)	P value	aOR	(95% CI)	P value
Model 1*									
Plasma BNP level									
BNP (log scale)	0.758	0.602–0.954	0.018	0.697	0.516–0.942	0.019	1.228	0.930–1.621	0.148
Estimated LV filling pressure									
Likely normal (lateral E/e' <8)		Reference			Reference			Reference	
Intermediate (lateral E/e' 8–12)	1.129	0.777–1.640	0.526	1.780	1.123–2.822	0.014	1.175	0.772–1.788	0.451
Likely high (lateral E/e' >12)	0.625	0.246–1.590	0.324	2.213	0.981–4.990	0.056	1.051	0.447–2.474	0.909
P for trend	0.976	0.722–1.320	0.875	1.623	1.156–2.277	0.005	1.104	0.797–1.529	0.553
Lateral E/e' (log scale)	1.112	0.640–1.933	0.706	3.364	1.670–6.777	0.001	1.384	0.738–2.596	0.311
Model 2**									
Plasma BNP level									
BNP (log scale)	0.758	0.602–0.954	0.018	0.689	0.509–0.922	0.016	1.223	0.926–1.615	0.155
Estimated LV filling pressure									
Likely normal (lateral E/e' <8)		Reference			Reference			Reference	
Intermediate (lateral E/e' 8–12)	1.169	0.776–1.761	0.454	1.418	0.858–2.343	0.173	1.086	0.687–1.717	0.723
Likely high (lateral E/e' >12)	0.658	0.250–1.732	0.397	1.578	0.666–3.740	0.300	0.932	0.379–2.291	0.879
P for trend	0.999	0.714–1.399	0.997	1.334	0.913–1.949	0.136	1.026	0.791–1.844	0.889
Lateral E/e' (log scale)	1.241	0.648–2.379	0.515	2.328	1.026–5.279	0.043	1.227	0.588–2.563	0.585
Model 3†									
Plasma BNP level									
BNP (log scale)	0.711	0.560–0.902	0.005	0.663	0.484–0.909	0.011	1.204	0.901–1.609	0.209
Estimated LV filling pressure									
Likely normal (lateral E/e' <8)		Reference			Reference			Reference	
Intermediate (lateral E/e' 8–12)	1.120	0.765–1.638	0.560	1.723	1.074–2.765	0.024	1.093	0.711–1.681	0.686
Likely high (lateral E/e' >12)	0.567	0.219–1.465	0.241	1.892	0.812–4.408	0.140	0.877	0.364–2.110	0.769
P for trend	0.952	0.699–1.297	0.756	1.534	1.081–2.178	0.017	1.019	0.728–1.425	0.914
Lateral E/e' (log scale)	1.068	0.603–1.894	0.821	2.881	1.390–5.973	0.004	1.157	0.602–2.224	0.662

*Model 1 adjusted for age and sex. **Model 2 adjusted for age, sex, and LV diastolic dysfunction. †Model 3 adjusted for age, sex, body mass index, regular smoking, regular alcohol intake, hypertension, dyslipidemia, diabetes mellitus, systolic blood pressure, eGFR, plasma BNP level (log scale) (except for BNP) and lateral E/e' (log scale) (except for estimated LV filling pressure). Abbreviations as in Table 1.

that the DD+ group may have higher LVFP than the DD− group despite having similar levels of plasma BNP. Because the LVMI was significantly larger in the DD+ group (86 (74–101) vs. 91 (76–106) g/m^2, P=0.009), we next investigated which LV geometry was more strongly associated with LV diastolic dysfunction. The number of patients with each LV geometry (normal, concentric remodeling, CH, eccentric hypertrophy) was 490 (52.1%), 204 (21.7%), 105 (11.2%), and 141 (15.0%), respectively. Thus, DD+ patients had a significantly greater proportion of CH (37 (7.9%) vs. 68 (14.5%); P=0.008).

Based on these results suggesting that the DD+ group had higher estimated LVFP relative to their plasma BNP levels, we examined the correlation between lateral E/e', a measure of estimated LVFP, and plasma BNP levels in each group. BNP levels and lateral E/e' were not significantly correlated in the DD+ group (r=0.062, P=0.183), but were significantly correlated in the DD– group (r=0.187, P<0.001) (**Figure 2**). According to the distribution of LV geometries in LV diastolic dysfunction patients, the CH group tended to have a lower increase in BNP levels for the increase in lateral E/e' (**Figure 2**).

We performed further analysis using ANCOVA to assess the effect of CH on the correlation between lateral E/e' and plasma BNP levels in both DD− and DD+ groups. Our analysis showed that in the DD− group, the effect of CH on this association was not significant (P=0.141). Conversely, in the DD+ group, the association between lateral E/e' and plasma BNP levels in CH was significantly different from other geometries (P=0.008), suggesting that the presence of CH may contribute to an increase in lateral E/e' without a corresponding increase in BNP level.

Accordingly, our data suggested that the DD+ group may include more subjects with relative NP deficiency who may develop CH.

Association of CH With Lower BNP and Higher Estimated LVFP

For an in-depth analysis of the association between plasma

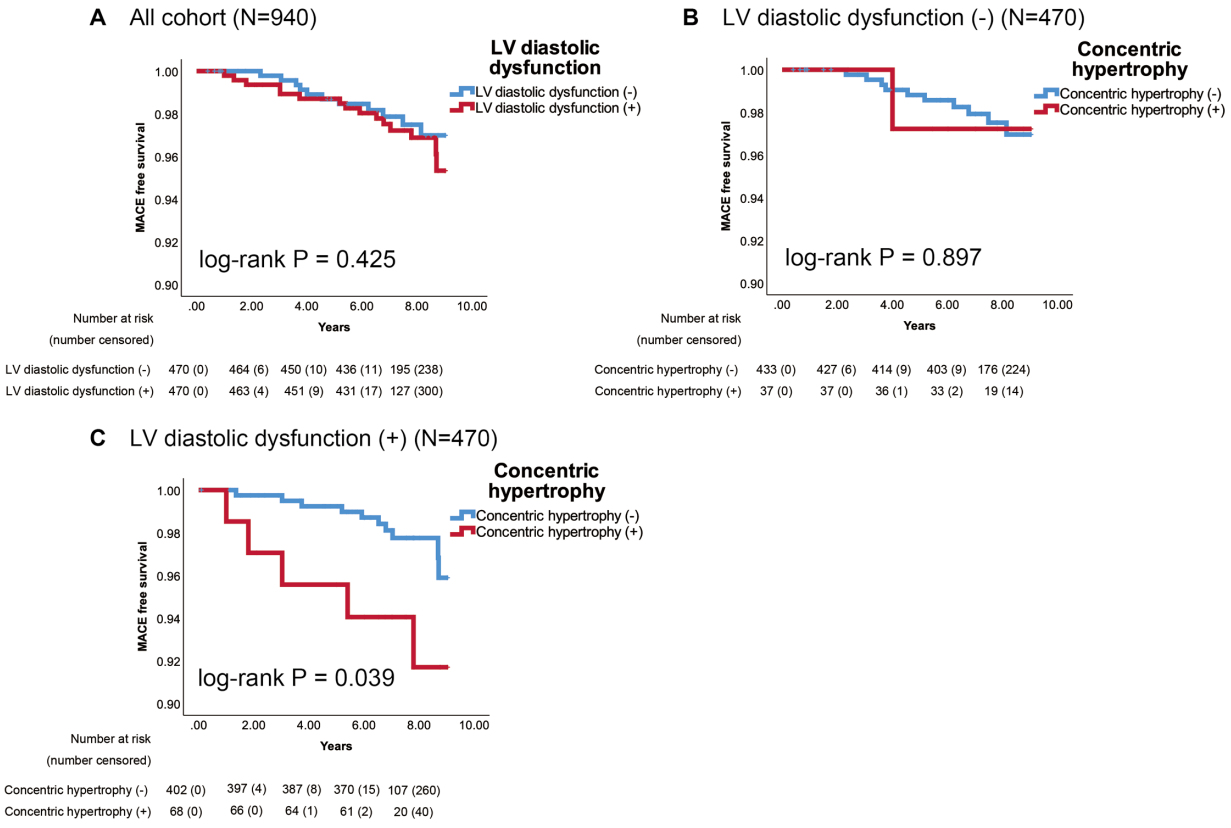

Figure 3. Kaplan-Meier analysis of LV diastolic dysfunction and concentric hypertrophy. (**A**) Kaplan-Meier analysis for MACE (composite endpoint of cardiovascular death, occurrence of acute myocardial infarction and stroke, hospitalization due to heart failure, and ischemic cardiovascular events) showed that the presence of LV diastolic dysfunction did not stratify the risk of MACE (log-rank P=0.425). (**B**) In the population without LV diastolic dysfunction (N=470), the presence of LV concentric hypertrophy did not stratify the risk of MACE (log-rank P=0.897). (**C**) In patients with LV diastolic dysfunction (N=470), LV concentric hypertrophy was associated with higher rates of MACE (log-rank P=0.039). LV, left ventricle; MACE, major adverse cardiovascular events.

BNP level, estimated LVFP, and LV geometry, we performed a multivariable polytomous logistic regression analysis in comparison with standard LV geometry that served as a reference. As shown in **Table 3**, model 1 (adjusted for age and sex) showed that increased BNP levels were associated with reduced odds of LV concentric remodeling and CH vs. normal geometry (aOR 0.758; 95% CI 0.602–0.954; P=0.018, and aOR 0.697; 95% CI 0.516–0.942; P=0.019, respectively). These results remained significant even in model 2 (additionally adjusted for LV diastolic dysfunction) (aOR 0.758; 95% CI 0.602–0.954; P=0.018, and aOR 0.689; 95% CI 0.509–0.922; P=0.016, respectively) and model 3 (additionally adjusted for BMI, regular smoking, regular alcohol intake, hypertension, dyslipidemia, diabetes mellitus, systolic blood pressure, systolic blood pressure, eGFR, and lateral E/e') (aOR 0.711; 95% CI 0.560–0.902; P=0.005, and aOR 0.663; 95% CI 0.484–0.909; P=0.011, respectively). BNP levels were not associated with the odds of LV eccentric hypertrophy in any of the 3 models. These results indicated that the odds of LV concentric remodeling and CH decreased significantly with increasing BNP levels, suggesting that a lower plasma BNP level is associated with an increased risk of concentric remodeling and hypertrophy. Moreover, model 1 showed that the increment of lateral E/e', an index of estimated LVFP, was associated with increased odds of CH vs. normal geometry (aOR 3.364; 95% CI 1.670–6.777; P=0.001); this also remained significant in the model 2 (aOR 2.328; 95% CI 1.026–5.279; P=0.043) and model 3 (aOR 2.881; 95% CI 1.390–5.973; P=0.004). In any of the 3 models, lateral E/e' was not associated with the odds of LV concentric remodeling or LV eccentric hypertrophy vs. normal geometry. This trend was maintained, although only model 2 lost statistical significance when LVFP was evaluated as a categorical variable according to the algorithm.[13] Namely, CH was simultaneously associated with lower BNP levels and higher estimated LVFP, suggesting that CH is most closely related to the state of relative BNP deficiency in the subclinical population without heart disease.

CH With Diastolic Dysfunction Predicts Poor Prognosis

Finally, we investigated the association between prognosis, LV diastolic dysfunction, and CH to reveal the utility of early detection of CH in a subclinical population with a relative deficiency of BNP. During the follow-up period of 7.8 (7.0–9.0) years, 26 (2.8%) patients developed MACE, including cardiovascular death in 1 (3.8%), acute myocardial infarction in 3 (11.5%), hospitalization for HF in 5

Table 4. Cox Hazards Regression Analysis for Major Adverse Cardiovascular Events

	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Age, 1 year	1.132 (1.068–1.200)	<0.001	1.122 (1.052–1.195)	<0.001
Male	2.596 (1.178–5.720)	0.018	2.997 (1.342–6.691)	0.007
Diastolic dysfunction and Concentric hypertrophy	3.072 (1.158–8.149)	0.024	3.272 (1.215–8.809)	0.019

All variables were included into the multivariate model. CI, confidence interval; HR, hazard ratio.

(19.2%), ischemic cardiovascular events in 3 (11.5%), and stroke in 14 (53.8%) participants. LV diastolic dysfunction did not stratify the risk of MACE in the analysis using the Kaplan-Meier life table (log-rank P=0.425, **Figure 3A**). Next, we investigated the clinical implications of the detection of CH in populations with (**Figure 3B**) and without LV diastolic dysfunction (**Figure 3C**) because the intrinsic endocrine deficiency of BNP was associated with CH even after adjustment for the presence of LV diastolic dysfunction (**Table 3**). In the DD– group (N=470), the presence of CH did not stratify the risk of MACE (log-rank P=0.897, **Figure 3B**). In contrast, in the DD+ group (N=470), CH was associated with higher rates of MACE (log-rank P=0.039; **Figure 3C**). **Table 4** shows the results of Cox proportional hazards analyses for predicting MACE. Univariate analysis revealed that CH with diastolic dysfunction was a significant predictor, and multivariate analysis showed that it was a significant independent predictor of MACE [hazard ratio (HR) 3.072 (95% CI:1.158–8.149, P=0.024), and HR 3.272 (95% CI 1.215–8.809, P=0.019) respectively]. These results suggested the importance of early identification of CH in the subclinical population, which appears to be associated with relative BNP deficiency and has a poor prognosis when complicated with diastolic dysfunction.

Discussion

Major findings in the current study are (1) subjects with LV diastolic dysfunction had a higher estimated LVFP and a higher prevalence of CH, which were not accompanied by elevated plasma BNP levels, than those without LV diastolic dysfunction; (2) both high estimated LVFP and low plasma BNP levels were independent factors associated with CH; and (3) CH with LV diastolic dysfunction predicted a poor prognosis. Unlike the well-known positive association between plasma BNP levels and LVH in HF patients and hypertension,[3,4] we found a negative association between plasma BNP levels and LVH in a subclinical population without heart disease, which might indicate a predisposition to diastolic dysfunction.

Relative Deficiency of BNP in a Subclinical Population

The relationship between LVH and NP levels is well established, and many studies have shown that plasma BNP levels are higher in patients with essential or untreated hypertension and CH.[3,4] These findings reflect the compensatory release of BNP from the heart to relieve hemodynamic stress. In contrast, we demonstrated that CH was associated with low BNP levels and high estimated LVFP in participants of a health checkup program, including many non-hypertensive subjects, by comparing subjects with and without diastolic dysfunction matched by noncardiac factors that affect plasma BNP levels. These findings suggested that a proportion of individuals attending health checkup programs have relative BNP deficiency, and are consistent with recent studies demonstrating cases of patients with insufficiently elevated NP concentrations, even when plasma BNP levels were expected to be elevated, such as hospitalization for HF or having abnormal cardiac structure or function. However, in none of the cases were there identifiable factors associated with low BNP levels, such as male sex, obesity, or African-American race.[5,16]

Characteristics of Relative Endocrine Deficiency of BNP

Few studies have epidemiologically evaluated NP deficiency before the onset of HF. We recently reported that lower plasma BNP levels are associated with LV concentric remodeling, which may be a precursor to LVH, as well as with insulin resistance in a subclinical population without cardiac disease and plasma BNP levels <35 pg/mL.[11] Those results supported that BNP deficiency due to decreased "basal" intrinsic BNP secretion may predispose to concentric LV remodeling when "additional" intrinsic BNP secretion by myocardial mechanical loading is absent. In the current study, we performed a more detailed analysis by including the subjects with plasma BNP levels >35 pg/mL, including the "additional" BNP secretion, and clarified that lower plasma BNP was associated with not only LV concentric remodeling but also CH. By binding to the NPRA, NPs exert pleiotropic effects, such as antihypertrophic, antifibrotic, and antidiabetic effects, by generating intracellular cyclic guanosine monophosphate (cGMP), followed by a cGMP-dependent protein kinase cascade.[1,20] Indeed, inadequate NP/cGMP signaling has been shown to predispose mice to LVH and abnormal glucose tolerance in mouse models.[7,21] However, previous clinical data have not demonstrated an association between lower NP levels and LVH, although abnormal glucose tolerance and diabetes mellitus have been reported.[22,23]

Mechanism of Endocrine Deficiency of BNP

Genetics plays a role in the endocrine deficiency of NPs, which can be attributed to the influence of genetic regions other than *NPPA*, *NPPB*, and *NPRA*.[24] We previously identified the DNA regulatory element, putative enhancer CR9, for *Nppa* and *Nppb*, which responds to mechanical loads by upregulating *Nppa* and *Nppb* expression in cardiomyocytes.[2] Mice with a deletion of the regulatory DNA region, including the CR9 element, show an endocrine deficiency in ANP and BNP.[7] Accordingly, unknown variants within the regulatory DNA regions may lead to endocrine deficiencies in NPs. We speculate that a relative deficiency of BNP against the estimated LVFP might be caused by genetic variation within the regulatory DNA region of *NPPA*/*NPPB*. Further studies focusing on the regulatory mechanisms of NP transcription are required to elucidate the endocrine deficiency of BNP.

Intervention for Intrinsic Endocrine Deficiency of BNP

CH is an independent risk factor for death, cardiovascular death, and cardiovascular events including congestive HF.[25] Subjects with diastolic dysfunction accompanied by CH had a worse prognosis than others in our cohort of subjects with no history of cardiovascular disease. Notably, CH was associated with lower BNP levels even after adjusting for LV diastolic dysfunction. In other words, a relative deficiency in BNP may be related to CH, even before the onset of diastolic dysfunction, and with a poor prognosis when diastolic dysfunction emerges as the disease progresses. Given the recent increase in the use of medicines that target the NP signaling pathway,[26,27] it is clinically significant to identify subjects with a relative NP deficiency for efficient treatment before the development of HF. LCZ696 (generic name: sacubitril valsartan sodium hydrate), a therapeutic agent that enhances the action of NP by inhibiting neprilysin, reduced the risk of cardiovascular death and HF hospitalization for patients with HF with preserved EF (HFpEF) by 13% compared to valsartan alone, although this difference was not statistically significant.[27] This finding suggests that some patients with HFpEF, such as those with a relative deficiency of NPs, could benefit from upregulating the NP signaling pathway, which further suggests the importance of detecting subjects with NP deficiency. Considering the results of our current study, it seems desirable to intervene in the NP-cGMP signaling pathway for CH before the onset of HF. Further investigation of the NP deficiency phenotype is warranted to define better the indications for therapeutic interventions targeting NP-cGMP signaling.

Study Limitations

First, some residual bias may still exist despite considering the background characteristics when adjusting for potential confounders in the multivariate analyses. In addition, propensity score matching was performed on non-cardiac factors that could affect blood levels of BNP after secretion to focus on BNP secretion, but this approach may not have been sufficient. Second, this cohort study included rural residents; therefore, the outcomes may differ from those of urban residents. Third, we did not know whether the LVFP estimated using Doppler echocardiography in the subclinical cohort was sufficiently reliable because of the lack of an invasive hemodynamic assessment. Finally, given that it was a subclinical population, the number of MACE was limited; therefore, it was impossible to analyze the prognosis following the present conclusion sufficiently.

Conclusions

Elevated estimated LVFP coupled with lower plasma BNP levels in a subclinical population without heart disease suggested that a relative deficiency of BNP is closely associated with CH. Furthermore, CH significantly correlated with poorer prognosis when accompanied by diastolic dysfunction. These findings underscore the potential clinical importance of early detection and treatment of BNP deficiency.

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Disclosures

O.T., S.I. are members of *Circulation Reports*' Editorial Team. The other authors declare that there are no conflicts of interest.

IRB Information

The study was approved by the institutional review board at National Cerebral and Cardiovascular Center and Arita-cho (M28-077-3, M14-025-6).

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