Table 3. Clinical Profiles of Twelve Hypertensive Patients with Missense/Frameshift Mutations in *HSD11β2* Gene

	Case											
	1	2	3	4	5	6	7	8	9	10	11	12
Polymorphism	L14F	L14F	L14F	L14F	L14F	R74H	R147H	R147H	R147H	T156I	4884Gdel	R335H
Age (years old)	73	71	64	51	59	70	76	69	85	78	75	67
Sex	male	female	male	female	male	male	male	male	male	male	female	female
BMI (kg/m^2)	21.39	20.45	20.20	24.09	30.30	27.92	24.03	22.12	26.17	21.69	29.97	21.50
Diagnosis	EHT, HL, HU, CRF, NIDDM, hypothyroidism	Renal HT, HL, CGN	EHT	ETH, HL	EHT, HL, obesity	EHT, HL, obesity	EHT, HU, OCI	EHT, HU, OCH, CRF	EHT, AF, AAA, obesity	EHT	RVHT, NIDDM, HL, obesity	EHT, HL
HT duration (years)	24	21	24	<1	9	15	19	20	21	8	30	41
HT initial onset age (years old)	49	50	40	—	50	55	57	49	64	70	45	26
HT family history	none	none	none	father	none	father, brother	mother, brother	none	none	mother	none	farther, mother, brother
SBP (mmHg)	138	136	152	140	130	140	134	138	154	134	170	148
DBP (mmHg)	70	80	88	68	80	86	72	70	84	68	90	80
Antihypertensive drugs	CCB, ARB	CCB, ACEI	CCB, BB, diuretics	CCB, BB, AB	CCB, ARB, BB	CCB, BB	CCB, AB	CCB, ACEI, AB	CCB	CCB, ACEI, AB	CCB, ACEI	CCB, BB
Na^+ (mEq/l)	141	141	140	142	140	141	141	140	143	143	140	139
K^+ (mEq/l)	4.4	5.2	4.1	4.2	4.2	3.6	4.2	5.2	4.5	4.2	4.6	5.0
Cl^- (mEq/l)	110	109	104	107	102	107	106	108	104	111	104	103
Creatinine (mg/dl)	2.7	0.8	0.6	0.5	0.6	1.1	1.3	2.9	1.2	0.8	0.6	0.8
Overt proteinuria	+	+	−	−	+	+	+	−	−	−	−	−
PRA (ng/ml/h)	3.8	0.9	6.3	0.1	0.5	2.9	1.9	no data	3.4	13.2	19.8	3.2
PAC (ng/dl)	8.8	8.5	no data	27.6	12.4	18.9	43.5	no data	7.7	14.6	7.0	14.1
FBS (mg/dl)	128	92	105	89	113	105	95	91	95	96	137	101
HbA1c (%)	6.0	5.6	5.4	5.2	5.6	6.0	5.1	5.2	5.1	5.0	8.7	5.7

BMI, body mass index; EHT, essential hypertension; HL, hyperlipidemia; HU, hyperuricemia; CRF, chronic renal failure; NIDDM, non-insulin dependent diabetes mellitus; HT, hypertension; CGN, chronic glomerulonephritis; OCI, old cerebral infarction; OCH, old cerebral hemorrhage; AF, atrial fibrillation; AAA, abdominal aortic aneurysma; RVHT, renovascular hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; CCB, calcium channel blocker; ARB, angiotension II receptor blocker; ACEI, angiotensin converting enzyme inhibitor; BB, β-adrenergic blocker; AB, α-adrenergic blocker; PRA, plasma renin activity; PAC, plasma aldosterone concentration; FBS, fasting blood sugar. Normal values in our institute: Na^+, 136–146 mEq/l; K^+, 3.6–4.9 mEq/l; Cl^-, 99–109 mEq/l; creatinine, 0.6–1.1 mg/dl; PRA, 0.2–2.7 ng/ml/h; PAC, 2–13 ng/dl.

Characteristics of Patients with Rare Missense/Frameshift Mutations in the Hypertensive Population

The characteristics of the 12 hypertensive patients who had missense/frameshift mutations (L14F, $n=5$; R74H, $n=1$; R147H, $n=3$; T156I, $n=1$; 4884Gdel, $n=1$; R335H; $n=1$) are shown in Table 3. Five patients out of the twelve had renal impairment including protein urea. Two (cases 1 and 2) of five patients with the L14F mutation had chronic renal failure (CRF) and chronic glomerulonephritis (CGN), and one (case 8) of three patients with the R147H mutation also had CRF. A patient with 4884Gdel (case 11) was diagnosed with renovascular hypertension caused by atherosclerosis with type 2 diabetes, hyperlipidemia and obesity (body mass index [BMI]: 29.97 kg/m^2). This patient was 75 years old, female, and had never smoked or drunk alcohol. This patient had microalbuminurea (urinary albumin excretion: 30.8 mg/g creatinine) without renal dysfunction (creatinine clearance: 112.5 ml/min) or cardiac hypertrophy (left ventricular mass index: 126.4 g/m^2). The average onset age of hypertension of the 12 patients with these missense mutations was 50.5 years. A patient with the R335H mutation (case 12) showed hypertension at her age of 26. Serum sodium levels of all patients were within normal range. There were no patients with hypokalemia as seen in AME.

Table 4. Basic Characteristics of Subjects in the General Population

	Women (n=1,946)	Men (n=1,709)
Age (years)	63.3±11.0	66.3±11.1*
Systolic blood pressure (mmHg)	128.0±19.7	131.8±19.4*
Diastolic blood pressure (mmHg)	76.5±9.8	79.7±10.7*
Body mass index (kg/m^2)	22.3±3.2	23.3±2.9*
Total cholesterol (mg/dl)	215.6±30.6*	197.9±30.3
HDL-cholesterol (mg/dl)	64.5±15.3*	55.0±14.1
Current smokers (%)	6.3	30.2†
Current drinkers (%)	29.6	67.2†
Present illness (%)		
Hypertension	38.0	47.3†
Hyperlipidemia	54.4†	27.8
Diabetes mellitus	5.2	12.8†

Values are expressed as mean±SD. Hypertension: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or antihypertensive medication; hyperlipidemia: total cholesterol ≥220 mg/dl or antihyperlipidemia medication; diabetes: fasting plasma glucose ≥126 mg/dl or non-fasting plasma glucose ≥200 mg/dl or HbA1c ≥6.5% or antidiabetic medication. *$p<0.05$ between women and men by Student t-test. †$p<0.05$ between women and men by χ^2 test. HDL, high-density lipoprotein.

Characteristics of Individuals with Rare Missense/Frameshift Mutations in the General Population

The characteristics of the 3,655 subjects comprising the Japanese general population group (1,709 men, 1,946 women) are summarized in Table 4. Age, SBP, DBP, BMI, percentage of current smokers, percentage of current drinkers, and prevalence of hypertension and diabetes mellitus were significantly higher in men than in women. Total cholesterol, high-density lipoprotein (HDL)–cholesterol, and percentage of hyperlipidemia were significantly higher in women than in men. In this population, 1,480 subjects were diagnosed with hypertension.

We successfully genotyped four genetic variations in the general population, which had a sample size of 3,655 individuals (2,175 normotensives and 1,480 hypertensives), but the genotyping failed for two of the genetic variations, T156I and 4884Gdel. In the general population, a missense mutation, R335H, was not present. The remaining three mutations, L14F, R74H, and R147H, were found in both hypertensive and normotensive subjects (Table 5). We identified 14 individuals with the L14F mutation. Six individuals with the L14F mutation had hypertension and eight were normotensive. We identified 20 individuals with the R74H mutation. Among them, eight showed hypertension and 12 were normotensive. We identified 8 individuals with the R147H mutation. Among them, three showed hypertension and five were normotensive. There were no statistically significant differences in any clinical characteristics between the subjects with the three missense mutations of *HSD11B2* and the subjects in the general population (Table 5).

Comparison of Missense/Frameshift Mutations in *HSD11B2* between Normotensives and Combined Hypertensives

As seen in Table 6, there was no difference in the prevalence of missense/frameshift mutations of *HSD11B2* between the combined subjects with hypertension and the normotensives.

Discussion

A missense mutation, P227L, in *HSD11B2* was previously identified in a patient with mild low-renin hypertension (*32*). This patient did not demonstrate the typical features of AME. The authors suggested that patients with mild low-renin hypertension may carry the mutations in the *HSD11B2* gene. In our study, we did not identify the P227L mutation in 953 Japanese hypertensives.

Genetic analyses of *HSD11B2* have been reported in two Japanese AME probands (*14*, *18*). In one family, the proband had a compound heterozygous mutation with a missense mutation, R208H, and a deletion of 3 nucleotides in codons 337–338 resulting in a substitution of Arg337 to His and a deletion of Tyr338 (CGCTAT to CAT: R337H and delta Y338) (*18*). Their family members, a father, mother, and elderly sister, who carried the heterozygous mutation were all normotensive and normokalemic, and had normal ratios of urinary [THF plus aTHF]/THE (THF, tetrahydrocortisol; aTHF, allotetrahydrocortisol; THE, tetrahydrocortisone). Another Japanese patient with AME had the homozygous missense mutation, S180F. The enzymatic activity of this mutant was 1.8% compared with the wild-type enzyme when cortisol was used as the substrate and 5.7% when corticosterone was used as the substrate (*14*). Figure 1 summarizes the reported polymorphisms in *HSD11B2*. In our study, none of the three causative genetic defects was identified, indicating that those mutations were not accumulated in the Japanese population.

We identified five novel missense mutations and one frameshift mutation in *HSD11B2* (Fig. 1, Table 2). As shown in Fig. 2A, five of the missense mutations occurred in residues that were highly conserved among the three different species, indicating that these mutations may result in functional changes in *HSD11B2*. However, neither hypertensive patients nor general subjects with these novel missense mutations showed any distinctive clinical characteristics during their health-check-ups.

We identified one hypertensive patient having renal artery stenosis with a frameshift mutation (4884Gdel) in *HSD11B2*. This deletion caused the frameshift at S219 with a premature stop codon at position 270 (Fig. 2B). A recent report indicated

Table 5. Accumulated Clinical Profiles of Subjects with Missense Mutations in *HSD11B2* in the General Population

	L14F	R74H	R147H
Number	14	20	8
Age (years old)	67.7±12.3	64.8±13.3	61.5±12.0
Sex (M/F)	7/7	9/11	5/3
Body mass index (kg/m^2)	23.4±4.0	22.4±2.9	23.9±1.7
Systolic blood pressure (mmHg)	125.4±23.0	128.7±23.4	124.9±19.9
Diastolic blood pressure (mmHg)	75.4±11.0	78.2±10.0	75.8±12.9
Total cholesterol (mg/dl)	213.9±34.0	213.8±37.0	199.3±36.4
HDL-cholesterol (mg/dl)	57.9±12.1	63.1±16.9	52.1±18.5
Triglyceride (mg/dl)	93.8±49.3	120.1±93.4	140.7±90.1
Creatinine (mg/dl)	0.8±0.2	0.7±0.2	0.8±0.2
Over proteinuria (yes/no)	1/13	0/20	0/8
FBS (mg/dl)	100.4±20.9	94.5±10.3	99.6±22.3
HbA1c (%)	5.7±0.8	5.4±0.7	5.6±0.9
Current smoker (yes/no)	2/12	4/16	1/7
Current drinker (yes/no)	5/9	9/11	4/4
Hypertension (yes/no)	6/8	8/12	3/5
Hyperlipidemia (yes/no)	10/4	11/9	6/2
Diabetes mellitus (yes/no)	6/8	2/18	2/6
Antihypertensive treatment (yes/no)	4/10	2/18	2/6

Values were expressed as mean±SD. Hypertension: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or antihypertensive medication; hyperlipidemia: total cholesterol ≥220 mg/dl or antihyperlipidemia medication; diabetes: fasting plasma glucose ≥126 mg/dl or non-fasting plasma glucose ≥200 mg/dl or HbA1c ≥6.5% or antidiabetic medication. M, male; F, female; HDL, high-density lipoprotein; FBS, fasting blood sugar.

Table 6. Number of Subjects with Missense/Frameshift Mutations in the Hypertensive and the General Populations

Mutations	Hypertensive population (n=953)	General population	
		Hypertensive subjects (n=1,480)	Normotensive subjects (n=2,175)
L14F	5	6	8
R74H	1	8	12
R147H	3	3	5
T156I	1	n.d.	n.d.
4884Gdel	1	n.d.	n.d.
R335H	1	0	0
Total	12	17	25

n.d., not determined.

that the heterozygous carriers with the defective allele of the *HSD11B2* gene showed essential hypertension (*16*). It is evident that this frameshift mutation results in the dysfunction of HSD11B2. The allele frequency of this mutation was very low (0.052%, 1 allele/1,906 alleles) in the Japanese hypertensive population. However, it is worth noting that this defective allele might be prevalent in other ethnic populations, because the frequency of some genetic mutations varies with ethnicity. Recently, rare genetic mutations collectively contributing to a quantitative trait variation, such as plasma levels of HDL-cholesterol, have been reported (*33*). We have performed large-scale sequence analyses of five hypertension candidate genes, *WNK4*, *SCNN1B*, *SCNN1G*, *NR3C2* and *RGS2*, to evaluate this hypothesis and found that a low but significant percentage of the hypertensive subjects had missense/frameshift mutations (*24–26*, *34*). Collectively, these rare mutations may make an at least partial contribution to hypertension.

The deduced NAD-binding sites reside in the conserved region from T82 to A111 (*2*), and the deduced catalytic site resides in the conserved region from Y232 to K236 (*35*). So far, more than ten genetic defects in patients with AME, most of whom had a severe deficiency of enzymatic activity confirmed by the expression analysis, have been reported and none of them overlap with the five missense mutations identified in the present study. Therefore, the effects on the HSD11B2 enzymatic activity of the mutations are not clear. In the future, an *in vitro* expression study should be performed to evaluate the activity of mutants and the ratios of urinary cortisol to cortisone metabolites in carriers of the mutations.

In the Caucasian population, a mutation at E178 that is synonymous with 553G>A which can be distinguished by *Alu* I restriction enzyme digestion, has been identified with a prevalence of 8.6% in the control subjects (*21*, *23*). This polymorphism was associated with end-stage renal disease but not with essential hypertension. We did not identify this polymor-

A

```
                    *
h-HSD    1 MERWPWPSGGAWLLVAARALLQLLRSDLRLGRPLLAALALLAALD   45
m-HSD    1 MERWPWPSGGAWLLVAARALLQLLRSDLRLGRPLLAALALLAALD   45
r-HSD    1 MERWPWPSGGAWLLVAARALIQLLRADLRLGRPLLAALALLAALD   45

                                          *
h-HSD   46 WLCQRLLPPPAALAVLAAAGWIALSRLARPQRLPVATRAVLITGC   90
m-HSD   46 WLCLRLMPPPAALVVLAGAGWIALSRLARPPRLPVATRAVLITGC   90
r-HSD   46 WLCQSLLPPSAALAVLAAAGWIALSRLARPQRLPVATRAVLITGC   90

                  *           *
h-HSD  136 QMDLTKPGDISRVLEFTKAHTTSTGLWGLVNNAGHNEVVADAELS  180
m-HSD  136 QMDLTKAEDISRVLEITKAHTASTGLWGLVNNAGLNIVVADVGLS  180
r-HSD  136 QMDLTKPADISRALEFTKAHTTSTGLWGLVNNAGHNDVVADVELS  180

                                 *
h-HSD  316 SDLTPVVDAITDALLAARPRRRYYPGQGLGLMYFIHYYLPEGLRR  360
m-HSD  316 PDLSPVVDAIIDALLAAQPRSRYYPGRGLGLMYFIHHYLPEGLRR  360
r-HSD  316 PDLSPVVDAITDALLAARPRPRYYPGRGLGLMYFIHYYLPEGLRR  360
```

B

Wild type

```
                    *
          ACTGTGGGGAGCCCAGCGGGGGACATGCCA
216       T  V  G  S  P  A  G  D  M  P       225
          TTCAAGACAGAGTCAGTGAGAAACGTGGGT
265       F  K  T  E  S     V  R  N  V  G    274
```

4884Gdel allele

```
          ACTGTGGGAGCCCAGCGGGGGACATGCCAT
216       T  V  G  A  Q  R  G  T  C  H       225
          TCAAGACAGAGTCAGTGAGAAACGTGGGTC
265       S  R  Q  S  Q     *  *  *
```

Fig. 2. *Partial amino acid sequence surrounding the mutations in* HSD11B2. *A: Alignment of partial amino acid sequences of HSD11B2 from two species and human HSD11B2. HSD11B2 sequences are from* Homo sapiens *(h),* Mus musculus *(m), and rabbit (r). Numbers indicate the position of amino acid sequence. The asterisks indicate the positions at which missense mutations occur (L14F, R74H, R147H, T156I, R335H) B: Nucleotide and amino acid sequences of wild-type allele and 4884Gdel allele. Numbers indicate the amino acid residues. An asterisk indicates the base deleted in the 4884Gdel allele, which causes a frameshift mutation from S218. This results in a 51–amino-acid extension that is is terminated by a stop codon (indicated by three asterisks).*

phism in our Japanese population.

In the Caucasian population, an intensive genetic study on the *HSD11B2* gene using 587 subjects, including 260 patients with end-stage renal disease, has been conducted, in which one missense mutation, L148V, and three synonymous mutations, T156, E178, and D388, were identified by the combination of single strand conformational polymorphism analysis and DNA sequencing (*36*). The results showed that allele frequencies did not differ significantly between control subjects and end-stage renal disease patients or between patients with hypertension and patients with end-stage renal disease. We did not identify these mutations in our Japanese population. Our results support their findings that the mutations in the *HSD11B2* gene do not affect hypertension.

In summary, we suggest that rare mutations in *HSD11B2*, L14F, R74H, R147H, T156I, R335H, and 4884Gdel may not collectively contribute to the pathogenesis of hypertension, although it was not clear whether abnormalities of electrolytes, renin activity, or aldosterone concentration were present, since our hypertensive patients with these missense/frameshift mutations were taking antihypertensive drugs. Further functional analyses of *HSD11B2* mutants are necessary to clarify the functional defects caused by these genetic variations in Japanese.

Acknowledgements

We would like to express our highest gratitude to Dr. Soichiro Kitamura, President of the National Cardiovascular Center, for his support of the Millennium Genome Project. We would also

like to express our gratitude to Drs. Otosaburo Hishikawa, Yasushi Kotani, and Katsuyuki Kawanishi and Mr. Tadashi Fujikawa for their continuous support of our population survey in Suita City. Finally, we would like to thank the members of the Satsuki-Junyukai and all the staff of the Division of Preventive Cardiology for their help in the medical examinations, and Ms. Y. Tokunaga for her technical assistance.

References

1. Agarwal AK, Monder C, Eckstein B, *et al*: Cloning and expression of rat cDNA encoding corticosteroid 11 beta-dehydrogenase. *J Biol Chem* 1989; **264**: 18939–18943.
2. Albiston AL, Obeyesekere VR, Smith RE, *et al*: Cloning and tissue distribution of the human 11beta-hydroxysteroid dehydrogenase type 2 enzyme. *Mol Cell Endocrinol* 1994; **105**: R11–R17.
3. Tannin GM, Agarwal AK, Monder C, *et al*: The human gene for 11 beta-hydroxysteroid dehydrogenase. Structure, tissue distribution, and chromosomal localization. *J Biol Chem* 1991; **266**: 16653–16658.
4. Agarwal AK, Mune T, Monder C, *et al*: Cloning of cDNA encoding an NAD^+-dependent isoform of 11beta-hydroxysteroid dehydrogenase in sheep kidney. *Endocr Res* 1995; **21**: 389–397.
5. Walker BR, Campbell JC, Williams BC, *et al*: Tissue-specific distribution of the NAD^+-dependent isoform of 11beta-hydroxysteroid dehydrogenase. *Endocrinology* 1992; **131**: 970–972.
6. New MI, Levine LS, Biglieri EG, *et al*: Evidence for an unidentified steroid in a child with apparent mineralocorticoid hypertension. *J Clin Endocrinol Metab* 1977; **44**: 924–933.
7. Geller DS: A mineralocorticoid receptor mutation causing human hypertension. *Curr Opin Nephrol Hypertens* 2001; **10**: 661–665.
8. Ferrari P: Genetics of the mineralocorticoid system in primary hypertension. *Curr Hypertens Rep* 2002; **4**: 18–24.
9. Dave-Sharma S, Wilson RC, Harbison MD, *et al*: Examination of genotype and phenotype relationships in 14 patients with apparent mineralocorticoid excess. *J Clin Endocrinol Metab* 1998; **83**: 2244–2254.
10. Ferrari P, Krozowski Z: Role of the 11beta-hydroxysteroid dehydrogenase type 2 in blood pressure regulation. *Kidney Int* 2000; **57**: 1374–1381.
11. Ferrari P, Lovati E, Frey FJ: The role of the 11beta-hydroxysteroid dehydrogenase type 2 in human hypertension. *J Hypertens* 2000; **18**: 241–248.
12. Mune T, Rogerson FM, Nikkila H, *et al*: Human hypertension caused by mutations in the kidney isozyme of 11beta-hydroxysteroid dehydrogenase. *Nat Genet* 1995; **10**: 394–399.
13. Li A, Tedde R, Krozowski ZS, *et al*: Molecular basis for hypertension in the "type II variant" of apparent mineralocorticoid excess. *Am J Hum Genet* 1998; **63**: 370–379.
14. Nunez BS, Rogerson FM, Mune T, *et al*: Mutants of 11beta-hydroxysteroid dehydrogenase (11-HSD2) with partial activity: improved correlations between genotype and biochemical phenotype in apparent mineralocorticoid excess. *Hypertension* 1999; **34**: 638–642.
15. Odermatt A, Dick B, Arnold P, *et al*: A mutation in the cofactor-binding domain of 11beta-hydroxysteroid dehydrogenase type 2 associated with mineralocorticoid hypertension. *J Clin Endocrinol Metab* 2001; **86**: 1247–1252.
16. Lavery GG, Ronconi V, Draper N, *et al*: Late-onset apparent mineralocorticoid excess caused by novel compound heterozygous mutations in the *HSD11B2* gene. *Hypertension* 2003; **42**: 123–129.
17. Quinkler M, Bappal B, Draper N, *et al*: Molecular basis for the apparent mineralocorticoid excess syndrome in the Oman population. *Mol Cell Endocrinol* 2004; **217**: 143–149.
18. Kitanaka S, Katsumata N, Tanae A, *et al*: A new compound heterozygous mutation in the 11beta-hydroxysteroid dehydrogenase type 2 gene in a case of apparent mineralocorticoid excess. *J Clin Endocrinol Metab* 1997; **82**: 4054–4058.
19. Wilson RC, Nimkarn S, New MI: Apparent mineralocorticoid excess. *Trends Endocrinol Metab* 2001; **12**: 104–111.
20. Ugrasbul F, Wiens T, Rubinstein P, *et al*: Prevalence of mild apparent mineralocorticoid excess in Mennonites. *J Clin Endocrinol Metab* 1999; **84**: 4735–4738.
21. Brand E, Chatelain N, Mulatero P, *et al*: Structural analysis and evaluation of the aldosterone synthase gene in hypertension. *Hypertension* 1998; **32**: 198–204.
22. Smolenicka Z, Bach E, Schaer A, *et al*: A new polymorphic restriction site in the human 11beta-hydroxysteroid dehydrogenase type 2 gene. *J Clin Endocrinol Metab* 1998; **83**: 1814–1817.
23. Lovati E, Ferrari P, Dick B, *et al*: Molecular basis of human salt sensitivity: the role of the 11beta-hydroxysteroid dehydrogenase type 2. *J Clin Endocrinol Metab* 1999; **84**: 3745–3749.
24. Kamide K, Takiuchi S, Tanaka C, *et al*: Three novel missense mutations of WNK4, a kinase mutated in inherited hypertension, in Japanese hypertensives: implication of clinical phenotypes. *Am J Hypertens* 2004; **17**: 446–449.
25. Kamide K, Tanaka C, Takiuchi S, *et al*: Six missense mutations of the epithelial sodium channel beta and gamma subunits in Japanese hypertensives. *Hypertens Res* 2004; **27**: 333–338.
26. Kamide K, Yang J, Kokubo Y, *et al*: A novel missense mutation, F826Y, in the mineralocorticoid receptor gene in Japanese hypertensives: implication of clinical phenotypes. *Hypertens Res* 2005; **28**: 703–709.
27. Matayoshi T, Kamide K, Takiuchi S, *et al*: The thiazide-sensitive Na^+-Cl^- cotransporter gene, *C1784T*, and adrenergic receptor-β3 gene, *T727C*, may be gene polymorphisms susceptible to the antihypertensive effect of thiazide diuretics. *Hypertens Res* 2004; **27**: 821–833.
28. Takiuchi S, Mannami T, Miyata T, *et al*: Identification of 21 single nucleotide polymorphisms in human hepatocyte growth factor gene and association with blood pressure and carotid atherosclerosis in the Japanese population. *Atherosclerosis* 2004; **173**: 301–307.
29. Kokubo Y, Inamoto N, Tomoike H, *et al*: Association of genetic polymorphisms of sodium-calcium exchanger 1 gene, NCX1, with hypertension in a Japanese general population. *Hypertens Res* 2004; **27**: 697–702.
30. Tanaka C, Mannami T, Kamide K, *et al*: Single nucleotide polymorphisms in the interleukin-6 gene associated with

blood pressure and atherosclerosis in a Japanese general population. *Hypertens Res* 2005; **28**: 35–41.
31. Tanaka C, Kamide K, Takiuchi S, *et al*: An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms. *Hypertens Res* 2003; **26**: 301–306.
32. Wilson RC, Dave-Sharma S, Wei JQ, *et al*: A genetic defect resulting in mild low-renin hypertension. *Proc Natl Acad Sci U S A* 1998; **95**: 10200–10205.
33. Cohen JC, Kiss RS, Pertsemlidis A, *et al*: Multiple rare alleles contribute to low plasma levels of HDL cholesterol. *Science* 2004; **305**: 869–872.
34. Yang J, Kamide K, Kokubo Y, *et al*: Genetic variations of regulator of G-protein signaling 2 in hypertensive patients and in the general population. *J Hypertens* 2005; **23**: 1497–1505.
35. Agarwal AK, Mune T, Monder C, *et al*: NAD^+-dependent isoform of 11beta-hydroxysteroid dehydrogenase. Cloning and characterization of cDNA from sheep kidney. *J Biol Chem* 1994; **269**: 25959–25962.
36. Zaehner T, Plueshke V, Frey BM, *et al*: Structural analysis of the 11beta-hydroxysteroid dehydrogenase type 2 gene in end-stage renal disease. *Kidney Int* 2000; **58**: 1413–1419.
37. Antonarakis SE, Nomenclature Working Group: Recommendations for a nomenclature system for human gene mutations. *Hum Mut* 1998; **11**: 1–3.

Uric Acid, Left Ventricular Mass Index, and Risk of Cardiovascular Disease in Essential Hypertension

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Abstract—Elevated serum uric acid (UA) is frequently encountered in individuals with hypertension, but whether the relationship between UA and cardiovascular events is circumstantial or causal remains to be answered. We examined the association between serum UA and left ventricular mass index (LVMI) and investigated prospectively whether the combination of UA and LVMI can predict the incidence of cardiovascular disease (CVD) in asymptomatic subjects with essential hypertension. A total of 619 subjects (mean age, 61 years; 52% female) free of prior CVD were included in this study. A significant association between UA and LVMI was also confirmed in multiple regression analysis (male: $F=4.29$, $P<0.04$; female: $F=4.24$, $P<0.05$). During follow-up (mean, 34 months), 28 subjects (14 female) developed CVD including myocardial infarction, angina pectoris, congestive heart failure, cerebral infarction, and transient cerebral ischemia. Sex-specific median values were used to separate the higher group from the lower group of UA and LVMI. Kaplan–Meier curves showed a significantly poorer survival rate in the group with higher UA and LVMI (LVMI, male: >126.9, female: >112.0 g/m^2; UA, male: >374.7, female: >303.3 μmol/L; log-rank $\chi^2=13.18$; $P<0.01$). Multivariate Cox regression analysis showed that the combination of higher UA and LVMI was an independent predictor for CVD events (hazard ratio, 2.38; $P<0.03$). Our findings demonstrate that UA is independently associated with LVMI and suggest that the combination of hyperuricemia combined with left ventricular hypertrophy is an independent and powerful predictor for CVD. The association between UA and CVD events may be introduced in part because of a direct association of UA with LVMI. (***Hypertension.* 2006;47:195-202.**)

Key Words: uric acid ■ cardiovascular diseases ■ hypertrophy ■ risk factors

Effective prevention of cardiovascular disease (CVD) requires the early detection and correction of predisposing conditions and risk factors in susceptible patients. Hypertension is a common risk factor for CVD, and the cardiovascular prognosis in patients with hypertension depends not only on the level of blood pressure (BP), but also on the presence of associated risk factors. Hyperuricemia is frequently encountered in hypertensive patients.[1] Several large epidemiologic studies have identified an association between increased serum uric acid (UA) and cardiovascular risk in the general population[2–6] and among patients with hypertension.[7,8] Other recent reports have also confirmed these associations by angiographic procedure.[9,10] Some studies have claimed that UA is an independent risk factor for CVD, whereas others have failed to identify UA as a significant and independent risk factor.[11–13] Thus, the status of UA as an independent risk marker remains controversial, and whether the relationship between UA level and cardiovascular events is circumstantial or causal remains to be answered.[2] On the other hand, the level of serum UA is affected by or linked to many factors, such as obesity, insulin resistance, dyslipidemia, and hypertension, all of which are also associated with left ventricular hypertrophy (LVH). In a recent report, in female subjects, UA level was independently associated with the presence of LVH detected by echocardiography.[14] These results suggest that UA level may be related to left ventricular mass index (LVMI).

In hypertension, LVH is initially a compensatory process against abnormal loading conditions, but it is also the first step toward the development of overt clinical disease, such as CVD.[15] In essential hypertension, the risk of future CVD complications is higher in patients with LVH on echocardiography than in those with normal left ventricular (LV) mass.[15,16] Thus, assessment of LV mass by echocardiography is a well-established procedure to estimate the risk of CVD in hypertensives.

The hypothesis that the combination of serum UA level and LVMI may be a strong predictor of CVD has never been examined. In this study, we investigated the relationship between UA level and LVMI in essential hypertensive subjects. Furthermore, we also examined prospectively the relations of UA level, LVMI, and their combination to the incidence of CVD during follow-up in asymptomatic hypertensive subjects.

Received September 26, 2005; first decision October 23, 2005; revision accepted December 5, 2005.

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Hypertension is available at http://www.hypertensionaha.org DOI: 10.116110.1161/01.HYP.0000200033.14574.14

Methods

Study Subjects

A total of 619 hypertensive subjects who had good-quality echocardiographic recordings were enrolled and monitored for 33.5±0.8 months in this study. All of the subjects were selected from patients who were admitted and underwent medical investigation at the National Cardiovascular Center in Osaka, Japan. Hypertension was defined as a systolic BP of ≥140 mm Hg and/or a diastolic BP of ≥90 mm Hg on repeated measurements or receiving antihypertensive treatment. Diabetes mellitus was defined according to the American Diabetes Association criteria.[17] Smoking was defined as current smoking or having a history of habitual smoking. Ischemic heart disease was defined as a ≥75% organic stenosis of ≥1 major coronary artery as confirmed by coronary angiography or a history of myocardial infarction or percutaneous transluminal coronary angioplasty. Renal insufficiency was defined as a serum creatinine concentration >176.8 μmol/L. All of the subjects enrolled in this study had essential hypertension. Exclusion criteria included ischemic heart disease, acute coronary syndrome, congestive heart failure (CHF; New York Heart Association class II or greater), chronic renal insufficiency, valvular heart disease, old cerebral infarction, and history of transient ischemic attack. Participants with moderate or severe aortic or mitral regurgitation or a heart rate >100 bpm were also excluded. The study protocol was approved by the ethics committee of our institution. All of the subjects enrolled in this study were Japanese, and all of the subjects gave informed consent to participate in this study.

Baseline Clinical Characteristics

After fasting overnight, BP was measured with an appropriate arm cuff and a mercury column sphygmomanometer on the left arm after a resting period of ≥10 minutes in the supine position. After BP measurements, venous blood sampling from all of the subjects was performed. Height and body weight were measured, and body mass index (BMI) was calculated. Insulin sensitivity was estimated using the homeostatic model assessment index; that is, plasma glucose level×(plasma insulin level/22.5). Urine samples were collected for 24 hours and used to evaluate creatinine clearance (Ccr). The following parameters were also determined: total cholesterol (T-chol), triglycerides (TG), high-density lipoprotein cholesterol (HDL-chol), serum UA, serum creatinine, and C-reactive protein (CRP) levels. Serum UA levels were determined by the uricase-peroxidase method.[18]

Echocardiographic Methods and Calculation of Derived Variables

Imaging and Doppler echocardiography were performed in all of the participants in this study. Studies were performed with phased-array echocardiography with M-mode, 2D, pulsed, and color-flow Doppler capabilities. LV internal dimension and septal and posterior wall thickness were measured at end-diastole and end-systole according to the American Society of Echocardiography recommendations.[19,20] Color-flow Doppler recordings were used to check for aortic and mitral regurgitation, as described previously.[21] End-diastolic dimensions were used to calculate LV mass by a previously reported formula.[22] LV mass was considered an unadjusted variable and was normalized by body surface area and expressed as LVMI.

The LV diastolic filling pattern was recorded from the apical transducer position with subjects in the left lateral decubitus position, with the sample volume situated between the mitral leaflet tips. The leading edge of the transmitral Doppler flow pattern was traced to derive the peak of early diastolic and atrial phase LV filling (E-velocity and A-velocity, respectively), their ratio (E/A ratio), and the deceleration time of early diastolic LV filling (DcT). All of the measurements were performed by a trained investigator who was blinded to the clinical data of the subjects.

Clinical End Points

For survival analysis, observation began on the date of echocardiography, with verified dates updated through March 2004. All of the subjects were followed at the National Cardiovascular Center in Osaka and treated by implementation of standard lifestyle and pharmacological measures. All of the subjects were periodically referred to our institution for BP control and other diagnostic procedures. CVD events of interest in this study were myocardial infarction and angina pectoris confirmed by electrocardiographic changes, coronary angiography and/or myocardial scintigraphy findings, stroke and transient cerebral ischemia confirmed by clinical symptoms, computed tomography and magnetic resonance angiography and/or cerebrovascular angiography findings, and CHF requiring hospitalization. CHF was diagnosed from clinical symptoms and findings (paroxysmal nocturnal dyspnea or cough, pulmonary rales because of pulmonary congestion, distended jugular veins, neck vein distension, enlarging heart size, pleural effusion and/or acute pulmonary edema on chest radiography, hepatojugular reflux, bilateral ankle edema, shortness of breath on ordinary exertion, and/or heart rate of ≥120 bpm). The cause of death was classified as CVD if there was sudden death from CVD by an independent review panel of physicians who were unaware of the echocardiographic and clinical findings. Events that were more equivocal, such as unrecognized myocardial infarction, angina pectoris, and transient cerebral ischemia, were not included as CVD for this analysis. Furthermore, patients with clinical evidence of pneumonia or uremia were excluded. For patients who experienced multiple nonfatal episodes of CVD, the analysis included only the first event.

TABLE 1. Baseline Clinical Characteristics of Study Subjects

Variables	Male	Female
n	296	323
Age, y	60.2±0.7	62.5±0.7*
BMI, kg/m²	24.6±0.2	24.2±0.2
Duration of hypertension, y	14.7±0.6	14.8±0.6
Smoking, %	72.0	18.7†
Systolic BP, mm Hg	142.5±0.9	144.6±0.8
Diastolic BP, mm Hg	83.2±0.6	80.8±0.6†
Pulse pressure, mm Hg	59.3±0.8	63.8±0.7†
Heart rate, bpm	66.4±0.5	67.4±0.5
Diabetes, %	26.7	18.9*
T-chol, mmol/L	5.16±0.04	5.37±0.04†
TG, mmol/L	1.69±0.06	1.29±0.06†
HDL-chol, mmol/L	1.24±0.02	1.43±0.02†
UA, μmol/L	378.5±4.8	313.2±4.6†
Ccr, Ml/min	101.6±2.3	94.2±2.2*
HOMA-index	1.79±0.10	1.63±0.10
CRP, mg/L	2.1±0.4	1.5±0.4
Septal wall thickness, mm	11.2±0.1	10.2±0.1†
Posterior wall thickness, mm	11.1±0.1	10.2±0.1†
LV internal diameter, mm	47.1±0.2	43.8±0.2†
LVMI, g/m²	130.5±1.7	116.0±1.7†
Peak E-velocity, m/s	0.68±0.01	0.72±0.01†
Peak A-velocity, m/s	0.77±0.01	0.86±0.01†
DcT, ms	229.3±2.8	230.1±2.6
E/A ratio	0.92±0.02	0.87±0.02*

HOMA indicates homeostatic model assessment. Data are mean±SE.
*$P<0.05$ and †$P<0.01$ vs male subjects.

Statistical Analysis

Parametric data are presented as mean±SE. The relations between LVMI or serum UA and various parameters were assessed using univariate linear regression analysis and Pearson's correlation coefficient. Multiple linear regression analysis was applied to identify independent determinants of LVMI after adjustment for potential confounding factors affecting LVMI.

Serum UA level and LVMI were stratified into 4 groups according to median values of baseline serum UA level and LVMI by each sex. One-way ANOVA with Dunnett multiple comparison posttest was used to analyze data among 4 groups. Event-free survival analysis was performed with the Kaplan–Meier method to plot the cumulative incidence of CVD, and the groups were compared by the Mantel log rank test. Cox proportional hazard analysis was used to examine the association between variables and the cumulative incidence of CVD. With respect to serum UA and LVMI, the cumulative incidence of CVD was calculated using the group with lower UA and LVMI as a reference for each other. These effects were measured by hazard ratios (HRs) and their 95% CIs based on Cox regression models. We used multivariable Cox proportional hazards regression models to examine the relations of serum UA and LVMI to CVD events, after accounting for relevant variables using a P value of <0.05 as the selection criterion. A P value <0.05 was considered statistically significant. All of the calculations were performed using a standard statistical package (JMP 4.0, SAS Institute).

Results

Association Between UA and LVMI

The baseline clinical and biochemical characteristics of the study subjects, analyzed on the basis of sex, are shown in Table 1. UA level and LVMI were significantly higher in men than in women. At baseline, 78.5% of the study patients were taking antihypertensive drugs, and 21.5% were complying with lifestyle measures only. Diuretics, β-blockers, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, and calcium-channel blockers were used alone or in various combinations in 11.3%, 26.7%, 33.9%, and 62.8% of the study patients, respectively. In addition, 9.0% of the study subjects were taking urate-lowering medication (allopurinol and probenecid).

We first examined the simple correlations between serum UA and clinical variables after dividing the subjects into 2 groups according to sex (Table 2). In both male and female subjects, UA level was significantly associated with BMI, TG, HDL-chol, Ccr, and LVMI. In addition, only in female subjects, there was a significant association between UA level and CRP, smoking, taking diuretics, and taking urate-lowering medication.

The simple correlations between LVMI and clinical variables were also examined (Table 2). In male subjects, LVMI was significantly correlated with duration of hypertension, systolic BP, pulse pressure, heart rate, T-chol, UA, and CRP and was significantly higher in smokers and those taking urate-lowering medication. In female subjects, LVMI was significantly correlated with age, BMI, duration of hyperten-

TABLE 2. Simple Correlation Among Serum UA, LVMI, and Clinical Characteristics

	UA, μmol/L		LVMI, g/m²	
Characteristics	Male	Female	Male	Female
Age	0.10	0.04	0.10	0.16†
BMI	0.13*	0.17†	0.10	0.18†
Duration of hypertension	0.10	0.09	0.13*	0.13*
Smoking, yes vs no	381.0±5.7 vs 369.0±9.1	332.6±10.5 vs 309.2±5.1*	133.3±2.1 vs 123.5±3.4*	119.7±3.8 vs 115.2±1.8
Systolic BP	0.01	0.05	0.14*	0.26†
Diastolic BP	0.01	0.06	0.01	0.04
Pulse pressure	0.03	0.01	0.17†	0.26†
Heart rate	0.03	0.01	−0.21†	−0.12*
Diabetes, yes vs no	381.0±5.7 vs 368.5±9.3	322.0±10.5 vs 311.1±5.1	131.0±3.5 vs 130.4±2.1	128.5±3.7 vs 113.1±1.8†
T-chol	0.03	0.02	0.12*	0.01
TG	0.25†	0.32†	0.08	0.19†
HDL-chol	−0.19†	−0.27†	−0.10	−0.15†
Ccr	−0.14*	−0.19†	−0.11	−0.01
HOMA-index	0.11	0.11	0.04	0.18†
CRP	0.03	0.15†	0.13*	0.08
LVMI	0.15†	0.16†		
Taking diuretics, yes vs no	397.1±15.3 vs 375.5±5.1	353.3±12.7 vs 307.5±4.8†	132.0±5.7 vs 130.4±1.9	123.7±4.6 vs 114.8±1.7
Taking urate-lowering medication, yes vs no	385.9±12.5 vs 376.2±5.3	431.7±22.6 vs 308.6±4.5†	140.2±4.6 vs 128.9±1.9*	121.2±8.5 vs 115.8±1.7
Peak E-velocity	−0.04	−0.01		
Peak A-velocity	0.01	0.06		
DcT	0.08*	0.12*		
E/A ratio	−0.02	−0.05		

HOMA indicates homeostatic model assessment. Data indicate correlation coefficients and mean±SE.
*$P<0.05$.
†$P<0.01$.

sion, systolic BP, pulse pressure, heart rate, TG, HDL-chol, homeostatic model assessment index, and UA and was significantly higher in diabetics.

Multiple linear regression analysis was performed including age, duration of hypertension, BMI, systolic and diastolic BP, heart rate, T-chol, TG, HDL-chol, Ccr, CRP, smoking, and diabetes and revealed that UA was independently associated with LVMI in male and female subjects (Table 3). In addition, even after adjustment for taking diuretics and taking urate-lowering medication, UA was still independently associated with LVMI (male, F=4.831, P=0.0290; female, F=4.591, P=0.0330).

To exclude the effect of drugs on UA level, we next examined the association between UA and LVMI after excluding subjects receiving diuretics and urate-lowering medication (male; n=232, female; n=273). Even after excluding these subjects, a significant association between UA and LVMI was observed (male: r=0.16, female: r=0.17, P<0.01 respectively).

LVH was considered to be present when LVMI was >125 for men and >110 g/m^2 for women.[23] UA level was significantly higher in subjects with LVH (male, 383.4±6.2 versus 363.5±6.6; female, 323.5±6.2 versus 303.0±6.5 μmol/L, P<0.03 respectively). A significant association between UA and LVH was also confirmed in multiple regression analysis including age, duration of hypertension, BMI, systolic and diastolic BP, heart rate, T-chol, TG, HDL-chol, Ccr, CRP, smoking, and diabetes (male, 384.5±7.6 versus 363.7±8.0, F=4.3, P<0.04; female, 329.7±9.1 versus 302.0±10.5 μmol/L, F=5.8, P<0.02).

The association between LV diastolic function and UA level was examined, and a significant association between UA and DcT was observed (Table 2). On the other hand, UA was not significantly associated with E-velocity, A-velocity, and E/A ratio. It is well described that early diastolic relaxation decreases with increasing age.[24] In the present study, we also found that DcT had a significant positive relationship with age (male: r=0.36, female: r=0.30, P<0.01 respectively), but not heart rate (male: r=−0.05, female: r=−0.01) and body surface area (male: r=0.07, female: r=0.05). Even after adjustment for age, DcT was significantly related to UA level (male: F=4.34, P<0.04; female: F=3.99, P<0.05).

TABLE 3. Independent Determinants of LVMI by Each Sex in Multiple Linear Regression Analysis

	Male		Female	
Variables	F	*P* Value	F	*P* Value
Age	0.068	0.7946	9.410	0.0024
BMI	4.718	0.0309	1.903	0.1689
Duration of hypertension	1.489	0.2236	0.026	0.8711
Smoking	3.935	0.0485	0.204	0.6516
Systolic BP	6.362	0.0124	20.479	0.0001
Diastolic BP	0.086	0.7702	0.011	0.9150
Heart rate	8.872	0.0032	5.859	0.0162
Diabetes	0.007	0.9357	9.837	0.0019
T-chol	2.826	0.0942	3.071	0.0808
TG	1.182	0.2781	6.239	0.0131
HDL-chol	0.294	0.5881	0.498	0.4812
UA	4.285	0.0396	4.244	0.0403
Ccr	1.886	0.1710	5.516	0.0196
CRP	0.246	0.6206	0.468	0.4944
	R^2=0.162; F=3.095; P=0.0002		R^2=0.249; F=6.344; P<0.0001	

Predictive Value of Serum UA and LVMI for CVD

Because of the sex difference in serum UA levels and LVMI values, different median values for men and women were used to separate the higher group from the lower group in each variable. Demographic and hemodynamic data of the subjects grouped according to the median value of serum UA (male: 374.7; female: 303.3 μmol/L) and LVMI (male: 126.9; female: 112.0 g/m^2) in each sex. As a result, the total subjects were divided into 4 groups as follows; lower LVMI and UA, lower LVMI and higher UA, higher LVMI and lower UA, and higher LVMI and UA. The baseline clinical and biochemical characteristics of the study subjects are shown in Table 4. There was a trend toward higher age, longer duration of hypertension, higher systolic BP, higher pulse pressure, and lower heart rate with increasing LVMI. On the other hand, the groups with higher UA showed higher BMI and lower Ccr. In addition, the group with higher LVMI and UA showed significantly lower HDL-chol and Ccr than that with higher LVMI and lower UA. At the follow-up contact, the proportions of subjects treated with diuretics, alone or combined with other agents, during follow-up were 6.8%, 11.9%, 9.6%, and 16.6% (P<0.05 versus lower LVMI and UA), respectively, in the 4 groups. The proportions of subjects treated with urate-lowering medication were 3.7%, 9.8%, 13.2% (P<0.05 versus lower LVMI and UA), and 10.7%, respectively.

During the follow-up period, 28 patients (4.5%; 14 female) developed CVD. There were 11 subjects with CHF, 1 with myocardial infarction, 8 with angina pectoris, 7 with cerebral infarction, and 1 with transient cerebral ischemia. Serum UA level and LVMI were significantly higher in patients who developed CVD during the follow-up period than in event-free subjects (UA: 385.3±16.8 versus 341.8±3.6 μmol/L, LVMI: 139.5±5.8 versus 122.1±1.3 g/m^2, P<0.01, respectively). Life table analyses of CVD throughout the follow-up period according to the 4 groups of baseline serum UA and LVMI are plotted in Figure 1. These curves illustrate significantly poorer survival in the group with higher UA and LVMI.

We next performed Cox regression analysis to examine whether the influence of higher UA and LVMI on CVD events was independent of other risk factors. As shown in Table 5, the risk for CVD was significantly higher in the group with higher UA and LVMI compared with that with lower UA and LVMI (HR, 2.70). In addition, age, duration of hypertension, pulse pressure, and Ccr were also significantly associated with the incidence of CVD. In multivariate Cox regression analysis, the combination of serum UA level and LVMI was an independent predictor for CVD (HR, 2.38).

TABLE 4. Baseline Clinical Characteristics of Study Subjects

	Lower LVMI		Higher LVMI	
Variables	Lower UA	Higher UA	Lower UA	Higher UA
N	166	145	138	170
Male, %	50.6	45.1	50.0	45.3
Age, y	59.3±0.9§	59.5±1.0§	63.9±1.0†	62.8±0.9*
BMI, kg/m^2	23.5±0.3‡	24.7±0.3†	24.4±0.3*	25.2±0.3†
Duration of hypertension, y	12.3±0.8§	14.9±0.9	16.0±0.9†	16.3±0.8†
Smokers, %	41.4	42.4	45.6	47.7
Systolic BP, mm Hg	140.8±1.2§	141.4±1.3‡	146.1±1.3†	145.9±1.1†
Diastolic BP, mm Hg	82.0±0.8	82.1±0.9	82.8±0.9	81.1±0.8
Pulse pressure, mm Hg	58.9±1.0‡	59.3±1.1‡	63.3±1.1*	64.9±1.0†
Heart rate, bpm	68.6±0.7§	68.0±0.7‡	65.4±0.8†	65.8±0.7*
Diabetes, %	18.9	18.8	27.2	26.5
T-chol, mmol/L	5.29±0.06	5.37±0.06	5.26±0.06	5.17±0.06
TG, mmol/L	1.28±0.08	1.76±0.09†‡	1.35±0.09	1.56±0.08†
HDL-chol, mmol/L	1.43±0.03	1.33±0.03	1.38±0.03	1.23±0.03†§
UA, μmol/L	280±5	400±5†	287±6	406±5†§
Ccr, mL/min	102.8±3.1	96.8±3.3	102.9±3.4	89.8±3.1†‡
HOMA-index	1.80±0.26	1.93±0.27	1.59±0.27	2.05±0.24
CRP, mg/L	1.42±0.36	1.25±0.37	1.62±0.37	1.83±0.33
Septal wall thickness, mm	9.6±0.1§	9.9±0.1§	11.5±0.1†	11.8±0.1†
Posterior wall thickness, mm	9.8±0.1§	9.9±0.1§	11.3±0.1†	11.5±0.1†
LV internal diameter, mm	43.7±0.3§	43.4±0.3§	46.9±0.3†	47.4±0.3†
LVMI, g/m^2	99.9±1.6§	99.9±1.7§	143.1±1.7†	147.8±1.6†
Peak E-velocity, m/s	0.71±0.01	0.71±0.01	0.69±0.01	0.70±0.01
Peak A-velocity, m/s	0.78±0.01§	0.81±0.02	0.84±0.02†	0.84±0.01†
DcT, ms	223.7±3.7	226.1±3.9	230.0±4.0	237.4±3.6*
E/A ratio	0.95±0.02§	0.92±0.02	0.85±0.02†	0.85±0.02†
No. of CVD events	2	5	5	16

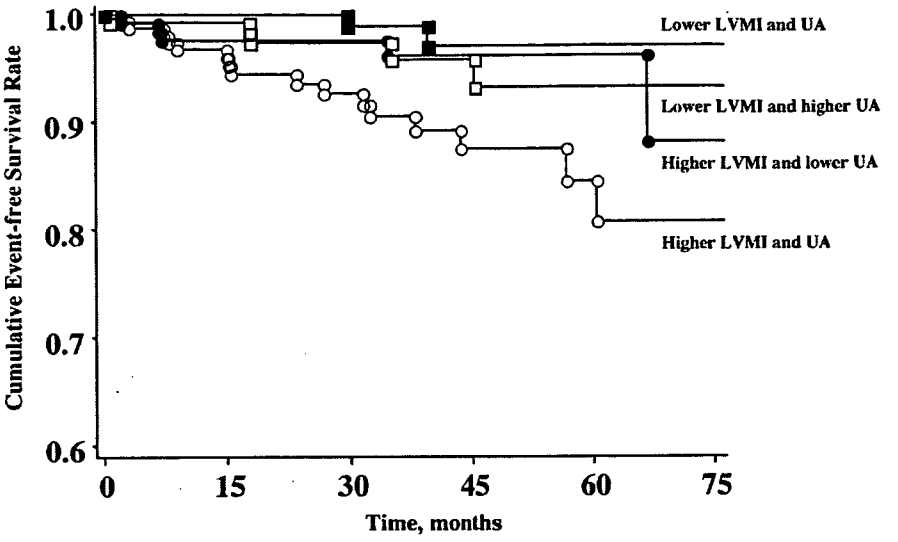
HOMA indicates homeostatic model assessment. Data are mean±E.
*$P<0.05$ and †$P<0.01$ vs lower LVMI and lower UA.
‡$P<0.05$ and §$P<0.01$ vs higher LVMI and lower UA.

In addition, the influence of the combination of UA and LVMI on CVD events was also examined by dividing the 4 groups according to the normal levels of UA (UA level ≤420 in men and ≤390 μmol/L in women) and with/without LVH; that is, normal UA and without LVH (n=244), hyperuricemia and without LVH (n=53), normal UA and LVH (n=245), and hyperuricemia and LVH (n=77). The independent predictive value of the hyperuricemia and LVH for CVD events was also confirmed by the Kaplan–Meier method (log-rank $\chi^2=5.58$; $P=0.0355$) and by Cox regression analysis (HR, 1.7; 95% CI, 0.78 to 3.41; $P<0.03$). In multivariate Cox regression analysis, the combination of hyperuricemia and LVH was an independent predictor for CVD events (HR, 1.8; 95% CI, 0.85 to 3.48; $P<0.05$).

Discussion

This study documented and validated that serum UA level is associated with LVMI, and the results of multiple linear regression analysis indicated that serum UA is independently associated with LVMI. Compared with the group with lower UA and LVMI, the group with higher UA and LVMI showed a condition of increased risk for cardiovascular and renal morbidity, such as significantly longer duration of hypertension, higher pulse pressure, worse dyslipidemia, and lower Ccr. Even after adjustment for other clinical factors, higher UA level and LVMI and age were independent predictors for CVD.

Our results suggest that serum UA is independently associated with LVMI, whereas an elevation of UA is associated with an actual metabolic disorder, and whether an elevation of serum UA level is the cause or result of LVH is unclear. The association between UA and LVMI might relate to an association of UA with other risk factors, especially including renal dysfunction, oxidative stress, severity of hypertension, and obesity. Renal dysfunction increases serum UA and activates the renin–angiotensin system, and angiotensin II is essential for the development of LVH.[25] UA is the final breakdown product of dietary or endogenous purines and is generated by xanthine oxidase (XO). A net release of urate in coronary heart disease[26] and the presence of XO in the human



Kaplan–Meier plots showing cumulative CVD-free survival in subjects according to 4 groups divided by median values of UA and LVMI (log-rank $\chi^2=13.18$; $P=0.0042$). Marker groups for LVMI (g/m^2): lower-LVMI, ≤126.9 for men and ≤112.0 for women; higher-LVMI, >126.9 for men and >112.0 for women. Marker groups for UA (μmol/L): lower-UA, ≤374.7 for men and ≤303.3 for women; higher-UA, >374.7 for men and >303.3 for women.

heart has been demonstrated.[27] UA may reflect the generation of superoxide and resultant oxidative stress via the XO system.[28] Furthermore, the independent association between UA and the severity of hypertension is well accepted.[1] On the other hand, there is a possibility that UA itself may induce LVH. Previous reports have shown that UA impaired NO generation and induced endothelial dysfunction and smooth muscle cell proliferation.[29,30] In experimental and in vitro systems, UA appears to have the ability to induce inflammatory mediators, such as tumor necrosis factor α,[31] and potentially stimulates mitogen-activated protein kinases,[32] which are known to induce cardiac hypertrophy.[33,34] These results suggest that cardiac hypertrophy may be, at least in part, attributable to an increase in UA itself, via stimulation of endothelial dysfunction, smooth muscle cell proliferation, and inflammation.

Our results showed that the incidence of CVD in subjects with higher UA and LVMI was ≈2.4-fold higher than that in subjects with lower UA and LVMI, even after adjustment for confounding factors. Thus, our results indicate that hyperten-

TABLE 5. Predictors for CVD Events by Cox Regression Analysis

	Univariate		Multivariate	
Variables, Unit of Increase	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
LVMI and UA	$\chi^2=12.79$	0.0051	$\chi^2=9.08$	0.0282
Lower LVMI and UA	1 (reference)		1 (reference)	
Lower LVMI and higher UA	1.01 (0.43 to 2.17)		1.14 (0.48 to 2.47)	
Higher LVMI and lower UA	1.02 (0.43 to 2.19)		1.01 (0.49 to 2.06)	
Higher LVMI and higher UA	2.70 (1.51 to 5.08)		2.38 (1.31 to 4.55)	
Age, 1 y	1.07 (1.03 to 1.12)	0.0004	1.05 (1.01 to 1.11)	0.0260
Sex, male	1.07 (0.74 to 1.56)	0.7212		
BMI, 1 kg/m^2	1.04 (0.93 to 1.16)	0.4566		
Duration of hypertension, 1 y	1.06 (1.03 to 1.10)	0.0003	1.03 (0.99 to 1.07)	0.0931
Smoking, yes	1.14 (0.78 to 1.66)	0.4844		
Systolic BP, 1 mm Hg	1.01 (0.98 to 1.03)	0.5092		
Diastolic BP, 1 mm Hg	1.03 (0.99 to 1.07)	0.0502		
Pulse pressure, 1 mm Hg	1.03 (1.00 to 1.05)	0.0343	1.01 (0.98 to 1.03)	0.5577
Heart rate, 1 bpm	0.98 (0.94 to 1.02)	0.4500		
Diabetes, yes	1.40 (0.93 to 2.05)	0.1005		
T-chol, 1 mmol/L	0.87 (0.52 to 1.43)	0.5757		
TG, 1 mmol/L	1.07 (0.69 to 1.34)	0.7261		
HDL-chol, 1 mmol/L	0.81 (0.28 to 2.07)	0.6685		
Ccr, 1 mL/min	0.99 (0.98 to 1.00)	0.0439	1.00 (0.99 to 1.01)	0.9661
HOMA-index, 1	1.01 (0.82 to 1.10)	0.8801		
CRP, 1 mg/L	1.00 (0.85 to 1.03)	0.7561		

HOMA indicates homeostatic model assessment.

sive subjects with LVH and hyperuricemia have an increased risk of developing CVD and suggest that the assessments of serum UA level and LVMI by echocardiography are useful and sensitive for predicting the risk for CVD. Many epidemiologic studies have attempt to identify whether hyperuricemia is an independent risk factor for CVD, but the results obtained were controversial after adjusting for other CVD risk factors, especially including LVH determined by electrocardiography.[7,8,13] Although hyperuricemia itself may have the ability to increase the risk of CVD, our results suggest that the association between UA and CVD events may be introduced in part because of a direct association of UA with LVMI. On the other hand, all of the antihypertensive drugs failed to show a cardioprotective effect in this study. Previous epidemiologic studies have also shown that UA level was independently predictive for the development of CVD even after antihypertensive treatment.[7,8,35,36] Furthermore, in the Systolic Hypertension in the Elderly Program trial, a subanalysis showed that the cardioprotection by diuretics was lost in those treated patients in whom UA levels increased.[36]

One notable result of this study is that, in the group with higher LVMI, the risk of CVD became higher with increasing UA level. This result may have been introduced because of decreased renal function and HDL-chol level, which are established risk factors for CVD, in subjects with hyperuricemia and LVH. Apart from renal function and lipid metabolism, there are other possible mechanisms by which the risk for CVD became higher with increasing UA levels. Several mechanisms have been proposed to account for the association between hyperuricemia and CVD, including the following: (1) the direct relationship of UA with severity of hypertension,[1] in which the predictive relationship of UA with BP is dose dependent;[37] (2) increased oxidative stress;[38] (3) a subtle reduction in glomerular filtration rate leading to impaired renal UA clearance;[39] (4) impaired NO production,[38] which activates the renin–angiotensin system[40] and induces endothelial dysfunction and smooth muscle cell proliferation;[29,30] (5) impaired platelet adhesiveness, disturbed hemorheology, and aggregation;[38] and (6) synthesis of monocyte chemoattractant protein-1 in vascular smooth muscle cells,[41] which is a chemokine that is importantly involved in CVD.[42] On the other hand, the close association between LVH and CVD events may be explained by decreased myocardial contractility, severe diastolic filling abnormalities, and increased oxygen requirement of the myocardium.[43] Our results showed that more severe relaxation impairment was observed in hyperuricemic subjects with LVH, and this "impaired relaxation" is known to be associated with increased risk of CVD.[44] In addition, a weak but significant association between UA and DcT, a marker of relaxation impairment, was observed in this study, and higher UA levels may contribute to the progression of LV dysfunction. Consequently, we propose the idea that, in subjects with LVH, severe hypertension, activation of oxidative stress and the renin–angiotensin system, stimulation of production of cytokines from leukocytes and chemokines from vascular smooth muscle cells, and more impaired relaxation may occur with increasing UA levels and enhance the risk for CVD.

The limitations of this study include missing baseline data and potentially important characteristics, such as menopause, alcohol intake, and a high-purine diet, which are also associated with a higher serum UA level. Because our data were obtained in subjects with treated essential hypertension at the start of the study, these results could underestimate the involvement of BP itself in the development of LVH and CVD events.

Perspectives

Our results demonstrate that UA is independently associated with LVMI and suggest that the combination of hyperuricemia with LVH is a powerful independent predictor for CVD. The association between UA and CVD events may be introduced in part because of a direct association of UA with LVMI. In hypertensive as well as LVH subjects, assessment of UA levels may help to refine CVD risk stratification. A crucial next step is to investigate whether UA is causally linked to LVH in a longitudinal setting. If so, hypouricemic agents might be used in clinical practice for LVH risk reduction in hypertensive patients. A large prospective population–based study will be important to confirm our preliminary observations.

Acknowledgments

We thank Chikako Tokudome for her secretarial assistance.

References

1. Johnson RJ, Feig DI, Herrera-Acosta J, Kang DH. Resurrection of uric acid as a causal risk factor in essential hypertension. *Hypertension.* 2005; 45:18–20.
2. Niskanen LK, Laaksonen DE, Nyyssonen K, Alfthan G, Lakka HM, Lakka TA, Salonen JT. Uric acid level as a risk factor for cardiovascular and all-cause mortality in middle-aged men: a prospective cohort study. *Arch Intern Med.* 2004;164:1546–1551.
3. Freedman DS, Williamson DF, Gunter EW, Byers T. Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I Epidemiologic Follow-up Study. *Am J Epidemiol.* 1995;141:637–644.
4. Fang J, Alderman MH. Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971–1992. National Health and Nutrition Examination Survey. *JAMA.* 2000;283:2404–2410.
5. Bengtsson C, Lapidus L, Stendahl C, Waldenstrom J. Hyperuricaemia and risk of cardiovascular disease and overall death. A 12-year follow-up of participants in the population study of women in Gothenburg, Sweden. *Acta Med Scand.* 1988;224:549–555.
6. Klein R, Klein BE, Cornoni JC, Maready J, Cassel JC, Tyroler HA. Serum uric acid. Its relationship to coronary heart disease risk factors and cardiovascular disease, Evans County, Georgia. *Arch Intern Med.* 1973; 132:401–410.
7. Verdecchia P, Schillaci G, Reboldi G, Santeusanio F, Porcellati C, Brunetti P. Relation between serum uric acid and risk of cardiovascular disease in essential hypertension. The PIUMA study. *Hypertension.* 2000; 36:1072–1078.
8. Alderman MH, Cohen H, Madhavan S, Kivlighn S. Serum uric acid and cardiovascular events in successfully treated hypertensive patients. *Hypertension.* 1999;34:144–150.
9. Short RA, Johnson RJ, Tuttle KR. Uric acid, microalbuminuria and cardiovascular events in high-risk patients. *Am J Nephrol.* 2005;25: 36–44.
10. Tuttle KR, Short RA, Johnson RJ. Sex differences in uric acid and risk factors for coronary artery disease. *Am J Cardiol.* 2001;87:1411–1414.
11. Staessen J. The determinants and prognostic significance of serum uric acid in elderly patients of the European Working Party on High Blood Pressure in the Elderly trial. *Am J Med.* 1991;90:50S–54S.
12. Wannamethee SG, Shaper AG, Whincup PH. Serum urate and the risk of major coronary heart disease events. *Heart.* 1997;78:147–153.

13. Culleton BF, Larson MG, Kannel WB, Levy D. Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. *Ann Intern Med.* 1999;131:7–13.
14. Viazzi F, Parodi D, Leoncini G, Parodi A, Falqui V, Ratto E, Vettoretti S, Bezante GP, Del Sette M, Deferrari G, Pontremoli R. Serum uric acid and target organ damage in primary hypertension. *Hypertension.* 2005; 45:991–996.
15. Casale PN, Devereux RB, Milner M, Zullo G, Harshfield GA, Pickering TG, Laragh JH. Value of echocardiographic measurement of left ventricular mass in predicting cardiovascular morbid events in hypertensive men. *Ann Intern Med.* 1986;105:173–178.
16. Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH. Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. *Ann Intern Med.* 1991;114:345–352.
17. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2003;26(Suppl 1):S5–S20.
18. Domagk GF, Schlicke HH. A colorimetric method using uricase and peroxidase for the determination of uric acid. *Anal Biochem.* 1968;22: 219–224.
19. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I, Silverman NH, Tajik AJ. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. Am Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr.* 1989;2: 358–367.
20. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation.* 1978;58:1072–1083.
21. Cooper JW, Nanda NC, Philpot EF, Fan P. Evaluation of valvular regurgitation by color Doppler. *J Am Soc Echocardiogr.* 1989;2:56–66.
22. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol.* 1986;57:450–458.
23. Hammond IW, Devereux RB, Alderman MH, Lutas EM, Spitzer MC, Crowley JS, Laragh JH. The prevalence and correlates of echocardiographic left ventricular hypertrophy among employed patients with uncomplicated hypertension. *J Am Coll Cardiol.* 1986;7:639–650.
24. Kitzman DW, Sheikh KH, Beere PA, Philips JL, Higginbotham MB. Age-related alterations of Doppler left ventricular filling indexes in normal subjects are independent of left ventricular mass, heart rate, contractility and loading conditions. *J Am Coll Cardiol.* 1991;18: 1243–1250.
25. Ichihara S, Senbonmatsu T, Price E Jr, Ichiki T, Gaffney FA, Inagami T. Angiotensin II type 2 receptor is essential for left ventricular hypertrophy and cardiac fibrosis in chronic angiotensin II-induced hypertension. *Circulation.* 2001;104:346–351.
26. De Scheerder IK, van de Kraay AM, Lamers JM, Koster JF, de Jong JW, Serruys PW. Myocardial malondialdehyde and uric acid release after short-lasting coronary occlusions during coronary angioplasty: potential mechanisms for free radical generation. *Am J Cardiol.* 1991;68:392–395.
27. Wajner M, Harkness RA. Distribution of xanthine dehydrogenase and oxidase activities in human and rabbit tissues. *Biochim Biophys Acta.* 1989;991:79–84.
28. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med.* 1985;312:159–163.
29. Rao GN, Corson MA, Berk BC. Uric acid stimulates vascular smooth muscle cell proliferation by increasing platelet-derived growth factor A-chain expression. *J Biol Chem.* 1991;266:8604–8608.
30. Khosla UM, Zharikov S, Finch JL, Nakagawa T, Roncal C, Mu W, Krotova K, Block ER, Prabhakar S, Johnson RJ. Hyperuricemia induces endothelial dysfunction. *Kidney Int.* 2005;67:1739–1742.
31. Netea MG, Kullberg BJ, Blok WL, Netea RT, van der Meer JW. The role of hyperuricemia in the increased cytokine production after lipopolysaccharide challenge in neutropenic mice. *Blood.* 1997;89:577–582.
32. Watanabe S, Kang DH, Feng L, Nakagawa T, Kanellis J, Lan H, Mazzali M, Johnson RJ. Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. *Hypertension.* 2002;40:355–360.
33. Yokoyama T, Nakano M, Bednarczyk JL, McIntyre BW, Entman M, Mann DL. Tumor necrosis factor-alpha provokes a hypertrophic growth response in adult cardiac myocytes. *Circulation.* 1997;95:1247–1252.
34. Sugden PH, Clerk A. "Stress-responsive" mitogen-activated protein kinases (c-Jun N-terminal kinases and p38 mitogen-activated protein kinases) in the myocardium. *Circ Res.* 1998;83:345–352.
35. Wang JG, Staessen JA, Fagard RH, Birkenhager WH, Gong L, Liu L. Prognostic significance of serum creatinine and uric acid in older Chinese patients with isolated systolic hypertension. *Hypertension.* 2001;37: 1069–1074.
36. Franse LV, Pahor M, Di Bari M, Shorr RI, Wan JY, Somes GW, Applegate WB. Serum uric acid, diuretic treatment and risk of cardiovascular events in the Systolic Hypertension in the Elderly Program (SHEP). *J Hypertens.* 2000;18:1149–1154.
37. Feig DI, Johnson RJ. Hyperuricemia in childhood primary hypertension. *Hypertension.* 2003;42:247–252.
38. Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S, Tuttle KR, Rodriguez-Iturbe B, Herrera-Acosta J, Mazzali M. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? *Hypertension.* 2003;41:1183–1190.
39. Vaziri ND, Freel RW, Hatch M. Effect of chronic experimental renal insufficiency on urate metabolism. *J Am Soc Nephrol.* 1995;6:1313–1317.
40. Eslami P, Corry DB, Nyby MD, Tuck ML. Inhibition of oxidative stress and improvement of nitric oxide production by ACE inhibitors and AT1 receptor blockers in uric acid stimulated vascular smooth muscle cells. *Am J Hypertens.* 2004;17(Suppl 1):S154–S155.
41. Kanellis J, Watanabe S, Li JH, Kang DH, Li P, Nakagawa T, Wamsley A, Sheikh-Hamad D, Lan HY, Feng L, Johnson RJ. Uric acid stimulates monocyte chemoattractant protein-1 production in vascular smooth muscle cells via mitogen-activated protein kinase and cyclooxygenase-2. *Hypertension.* 2003;41:1287–1293.
42. Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, Rollins BJ. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol Cell.* 1998;2: 275–281.
43. Devereux RB, Roman MJ, Palmieri V, Okin PM, Boman K, Gerdts E, Nieminen MS, Papademetriou V, Wachtell K, Dahlof B. Left ventricular wall stresses and wall stress-mass-heart rate products in hypertensive patients with electrocardiographic left ventricular hypertrophy: the LIFE study. Losartan Intervention For Endpoint reduction in hypertension. *J Hypertens.* 2000;18:1129–1138.
44. Schillaci G, Pasqualini L, Verdecchia P, Vaudo G, Marchesi S, Porcellati C, de Simone G, Mannarino E. Prognostic significance of left ventricular diastolic dysfunction in essential hypertension. *J Am Coll Cardiol.* 2002; 39:2005–2011.

Journal of the American College of Cardiology
© 2006 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 47, No. 4, 2006
ISSN 0735-1097/06/$32.00
doi:10.1016/j.jacc.2005.11.030

B-Type Natriuretic Peptide Strongly Reflects Diastolic Wall Stress in Patients With Chronic Heart Failure

Comparison Between Systolic and Diastolic Heart Failure

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OBJECTIVES We explored the stimulus for B-type natriuretic peptide (BNP) secretion in the clinical setting of heart failure (HF).

BACKGROUND Increasingly, plasma BNP levels are being incorporated into the clinical assessment and management of systolic heart failure (SHF) as well as diastolic heart failure (DHF). However, heterogeneity in BNP levels among individuals with HF can cause some confusion in interpreting results.

METHODS In 160 consecutive patients presenting with HF, we measured plasma BNP levels and performed echocardiography and cardiac catheterization. Systolic and diastolic meridional wall stress was calculated from echocardiographic and hemodynamic data.

RESULTS Although plasma BNP had a significant correlation ($r^2 = 0.296$ [$p < 0.001$]) with left ventricular end-diastolic pressure (EDP) as previously reported, the correlation between plasma BNP and end-diastolic wall stress (EDWS) ($r^2 = 0.887$ [$p < 0.001$]) was more robust. In a subanalysis of 62 patients with DHF, a similar result was obtained ($r^2 = 0.143$ for EDP and $r^2 = 0.704$ for EDWS). In a comparison between SHF and DHF, the BNP level was significantly higher in SHF ($p < 0.001$). Although EDP did not show any difference, EDWS was significantly higher in SHF than in DHF ($p < 0.001$).

CONCLUSIONS The present study shows that plasma BNP levels reflect left ventricular EDWS more than any other parameter previously reported, not only in patients with SHF, but also in patients with DHF. The relationship of left ventricular EDWS to plasma BNP may provide a better fundamental understanding of the interindividual heterogeneity in BNP levels and their clinical utility in the diagnosis and management of HF. (J Am Coll Cardiol 2006;47:742–8)

Plasma B-type natriuretic peptide (BNP) levels are reported not only to be a strong marker of left ventricular (LV) dysfunction, but also a marker to predict morbidity and mortality accurately in patients with chronic heart failure (HF) (1,2). Recently, BNP-guided therapy for chronic HF has been suggested. Troughton et al. (3) demonstrated that pharmacotherapy guided by BNP levels reduces cardiovascular events and delays time to first cardiovascular event compared with intensive clinically guided therapy. Recent reports also demonstrated the contribution of LV diastolic function to plasma BNP levels and the usefulness of BNP in the diagnosis of diastolic HF (4).

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However, heterogeneity in BNP levels among individuals with HF has been recognized, and it has caused some confusion in interpreting results (5). Previous human studies have suggested correlations between BNP levels and cardiac functional or dimensional indexes such as end-diastolic pressure (EDP), ejection fraction (EF), pulmonary capillary wedge pressure, and LV volume, none of which sufficiently explain the heterogeneity (6–9). Therefore, it is essential to determine the stimulus for BNP secretion in the clinical setting of HF. In vitro studies have clarified the mechanism of secretion and regulation of BNP precisely (10). Stretch of cardiomyocytes is reported to be the most important stimulus of BNP regulation (11). It is also believed that BNP in humans may be released from the heart in response to increased wall stress. However, there have been few human studies exploring a direct relationship between wall stress and BNP regulation (12). Vanderheyden et al. (13) have very recently demonstrated, for the first time, in 40 patients with aortic stenosis (AS), a significant correlation of BNP with LV end-diastolic wall stress (EDWS). In their study, however, subjects were limited to patients with AS. Hence, there now is a need for the same assessment in patients

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Manuscript received January 25, 2005; revised manuscript received July 13, 2005, accepted August 22, 2005.

Abbreviations and Acronyms

AS	= aortic stenosis
BNP	= B-type natriuretic peptide
CHF	= congestive heart failure
DHF	= diastolic heart failure
EDP	= end-diastolic pressure
EDWS	= end-diastolic wall stress
EF	= ejection fraction
HF	= heart failure
LV	= left ventricle/ventricular
LVEDVI	= left ventricular end-diastolic volume index
LVMI	= left ventricular mass index
SHF	= systolic heart failure
SWS	= systolic wall stress

with HF of various etiologies. Accordingly, in the present study, we evaluated plasma BNP levels in 160 consecutive patients presenting with HF of various etiologies including diastolic HF.

METHODS

Patients. Among the patients referred to our National Cardiovascular Center Hospital between October 2003 and December 2004, we included in this study those admitted with congestive heart failure (CHF) consecutively. Patients who did not undergo LV catheterization or had renal dysfunction (serum creatinine >2.0 mg/dl) were excluded. A sample of 160 patients was obtained. For all participants, cardiac catheterization and echocardiograms were performed at a compensated CHF stage (before discharge), and plasma BNP was measured on the day before cardiac catheterization. The clinical characteristics of these patients are listed in Table 1.

BNP assay. Blood was collected into tubes containing EDTA, and plasma BNP was measured using a validated and commercially available immunoassay kit (Tosoh Co. Ltd., Japan).

Cardiac catheterization. Left ventricular pressure was recorded with a 5-F pigtail catheter connected to a fluid-filled transducer. Left ventricular volume and EF were determined with left ventriculography with contrast medium using Kennedy's formula.

Echocardiography. Echocardiographic examinations were performed with a Sonos 5500 machine equipped with a 2.5-MHz probe. M-mode images were obtained to measure left atrial and ventricular dimensions (14). The left ventricular mass index (LVMI) was estimated from the formula of Devereux et al. (15). The severity of mitral regurgitation was quantified on a semicontinuous scale from none (0) to moderately severe (3+). In patients with sinus rhythm, the pulsed Doppler transmitral flow velocity was recorded to measure a ratio of peak mitral E-wave velocity to peak mitral A-wave velocity (E/A ratio) and the deceleration time of the mitral E-wave velocity.

On the basis of hemodynamic and echocardiographic data, end-diastolic and systolic meridional wall stresses (WS) were calculated. These were obtained by using the formula: $WS = 0.334 \times P(LVID)/WT(1 + WT/LVID)$, where P = LV pressure (i.e., peak systolic pressure or EDP, which was obtained during cardiac catheterization), LVID = left ventricular internal dimension, and WT = wall thickness (16). In the present study, the posterior wall thickness was used to assess WT regardless of regional wall motion abnormalities. In the analysis of the interobserver reproducibility of the posterior wall thickness measurement in 48 patients with CHF, a high degree of the reproducibility was

Table 1. Patient Characteristics

	Total	SHF	DHF	p Value
n	160	98	62	
Women	31	25	40	0.052
Age, yrs	66.8 ± 1.0	66.3 ± 1.3	67.7 ± 1.6	0.485
BMI, kg/m^2	22.9 ± 0.3	22.8 ± 0.4	23.1 ± 0.4	0.684
NYHA functional class ≥2	32	37	24	0.138
HT	71	61	87	0.001
DM	35	36	34	0.946
HLP	53	49	58	0.338
AF	18	17	19	0.912
Etiology				
DCM	18	30	0	
ISCM or OMI	29	44	6	
HHD	26	9	53	
VHD	26	17	40	
Medications				
ACEI or ARB	70	77	57	0.013
Beta-blocker	51	54	46	0.397
Diuretics	60	71	42	0.001
BNP, pg/ml	282 ± 23	379 ± 33	129 ± 13	<0.001

Values are mean ± SEM or %.
ACEI = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin receptor blocker; BMI = body mass index; DCM = dilated cardiomyopathy; DHF = diastolic heart failure; DM = diabetes mellitus; HHD = hypertensive heart disease; HLP = hyperlipidemia; HT = hypertension; ISCM = ischemic cardiomyopathy; NYHA = New York Heart Association; OMI = old myocardial infarction; SHF = systolic heart failure; VHD = valvular heart disease.

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Table 2. Echocardiographic and Hemodynamic Parameters

	Total (n = 160)	SHF (n = 98)	DHF (n = 62)	p Value
FS, %	27 ± 1	20 ± 1	38 ± 1	<0.001
LVEDD, mm	57 ± 1	61 ± 1	50 ± 1	<0.001
LVMI, g/m^2	166 ± 4	179 ± 5	145 ± 6	<0.001
LAD, mm	45 ± 1	45 ± 1	44 ± 1	0.779
E/A	1.3 ± 0.1	1.5 ± 0.2	1.0 ± 0.1	0.024
EF, %	41.5 ± 1.1	32.0 ± 0.9	56.4 ± 0.5	<0.001
LVEDVI, ml/m^2	106 ± 4	125 ± 15	76 ± 2	<0.001
LVSP, mm Hg	134 ± 3	124 ± 3	151 ± 4	<0.001
LVEDP, mm Hg	14.9 ± 0.4	15.0 ± 0.6	14.8 ± 0.5	0.829

Values are mean ± SEM.
EF = ejection fraction; E/A = ratio of peak mitral E-wave velocity to peak mitral A-wave velocity; FS = fractional shortening; LAD = left atrial dimension; LVEDD = left ventricular end-diastolic dimension; LVEDP = left ventricular end-diastolic pressure; LVEDVI = left ventricular end-diastolic volume index; LVMI = left ventricular mass index; LVSP = left ventricular peak systolic pressure. Other abbreviations as in Table 1.

found with an intraclass correlation coefficient value 0.830 (95% confidence interval 0.609 to 0.925), and absolute difference was small (mean ± SD; 0.01 ± 1.16 mm). Also, adequate M-mode images were not available in three patients, and they were excluded in the present study.

Statistical analysis. Comparisons between groups were made using chi-square analysis for proportions and unpaired Student *t* tests for continuous variables. Linearity of a relationship between two variables was assessed by linear regression analysis; $p < 0.05$ was considered significant. Results were expressed as mean ± SEM.

RESULTS

Patient characteristics. Clinical characteristics of the group of 160 patients are summarized in Table 1. Mean age was 66.8 ± 1.0 years (range 20 to 87 years), and 31% of the patients were women. In all, 98 patients had HF symptoms with an LV EF of ≤50%. These comprised the systolic heart failure group (SHF). The diastolic heart failure group (DHF) was comprised of 62 patients with preserved systolic function (LV EF >50%). Mean age and body mass index did not differ significantly between SHF and DHF groups, while there was a trend of more female patients in DHF. A history of hypertension and etiologies of dilated cardiomyopathy and ischemic cardiomyopathy/old myocardial infarction were more prevalent in SHF. Patients with SHF were more likely to be taking angiotensin-converting enzyme inhibitors or angiotensin receptor blockers and diuretics.

Geometric and functional parameters obtained by echocardiography or cardiac catheterization are shown in Table 2. In total patients, mean EF was 41.5 ± 1.1% (range 13% to 66%), and mean LVMI and LV end-diastolic volume index (LVEDVI) were 166 ± 4 g/m^2 and 106 ± 4 ml/m^2, respectively.

Correlations of plasma BNP to echocardiographic and hemodynamic parameters. Scatter plots of plasma BNP levels (dependent variable) against some echocardiographic and hemodynamic parameters (independent) are shown in Figure 1. There were strong correlations between LV EF, LVEDVI or LV end-systolic volume index, or LV EDP and plasma BNP (coefficient of correlation; $r^2 = 0.325$, 0.343, 0.421, and 0.328, respectively). There were weak correlations with parameters of transmitral Doppler flow $r^2 = 0.201$ and 0.101 for E/A and deceleration time, respectively. In contrast, LVMI and left atrial diameter did not show significant correlations with BNP levels. Although LV systolic wall stress (SWS) calculated by echocardiographic and hemodynamic parameters showed a modest correlation ($r^2 = 0.277$), a correlation of BNP with LV EDWS was much more robust ($r^2 = 0.887$).

Although age, gender, and atrial fibrillation were not significantly associated, body mass index (BMI) and New York Heart Association functional class ≥II were associated with BNP levels ($p < 0.001$ in both).

Comparison between SHF and DHF. Plasma BNP levels were significantly higher in SHF than in DHF (median [interquartile range]; 267 [136 to 583] and 105 [64 to 146] pg/ml, respectively, $p < 0.001$); however, EDP levels did not show any differences as shown in Figure 2 and Table 2. Other parameters such as SWS, EDWS, LV end-diastolic dimension, LVMI, LVEDVI, and LV peak systolic pressure were significantly higher in SHF than in DHF ($p < 0.001$). Scatter plots in patients with SHF and DHF are demonstrated in Figures 3A and 3B and Figures 3C and 3D, respectively. End-diastolic wall stress showed a better correlation with BNP ($r^2 = 0.704$) than EDP ($r^2 = 0.143$) in DHF as well as in SHF.

Subanalysis in patients without local wall motion abnormality. It is conceivable that this estimation of wall stress did not accurately reflect the entire non-uniform LV wall stress in patients with regional asynergy in LV wall motion or with variation in segmental LV wall thickness. In the present study, 83% of patients with ischemic cardiomyopathy or old myocardial infarction and 28% with dilated cardiomyopathy had regional wall motion abnormalities. Therefore, a subanalysis was performed for patients without local wall motion abnormality (n = 105). As a result, an even stronger correlation was obtained as shown ($r^2 = 0.919$). A correlation in patients with regional wall motion abnormality (n = 55) was still strong ($r^2 = 0.820$).

DISCUSSION

Heterogeneity of BNP levels among individuals with HF can cause some confusion in interpreting results. It has been unclear why some patients with LV EF <35% have BNP levels in the normal range whereas others exhibit extremely elevated levels, and why some patients with isolated diastolic dysfunction (i.e., with normal EF) show a similar increase of plasma BNP as do the patients with severe systolic dysfunction. One of the answers to the question has been the change of EDP levels in the LV (6). Another recent report has demonstrated that heterogeneity of BNP levels in patients with systolic HF reflects the severity of diastolic abnormality, right ventricular function, and mitral regurgi-

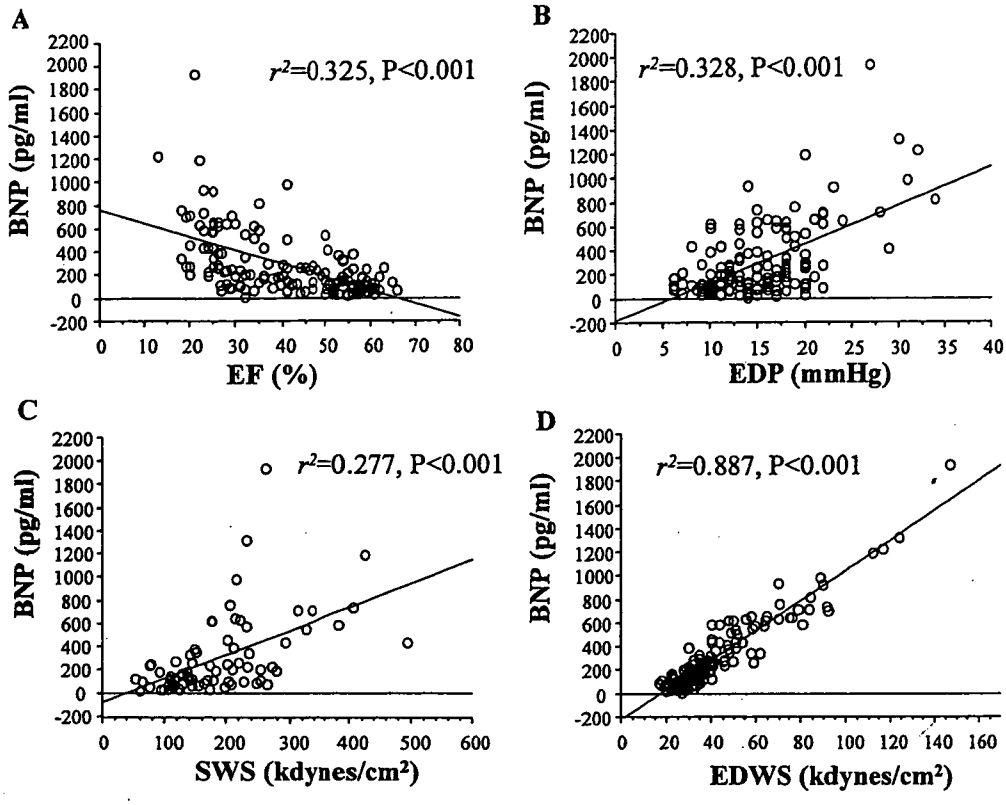

Figure 1. Correlation between B-type natriuretic peptide (BNP) and left ventricular functional parameters in all 160 patients. (A) Left ventricular ejection fraction (EF) (%). (B) End-diastolic pressure (EDP) (mm Hg). (C) End-systolic wall stress (SWS) (kdynes/cm^2). (D) End-diastolic wall stress (EDWS) (kdynes/cm^2).

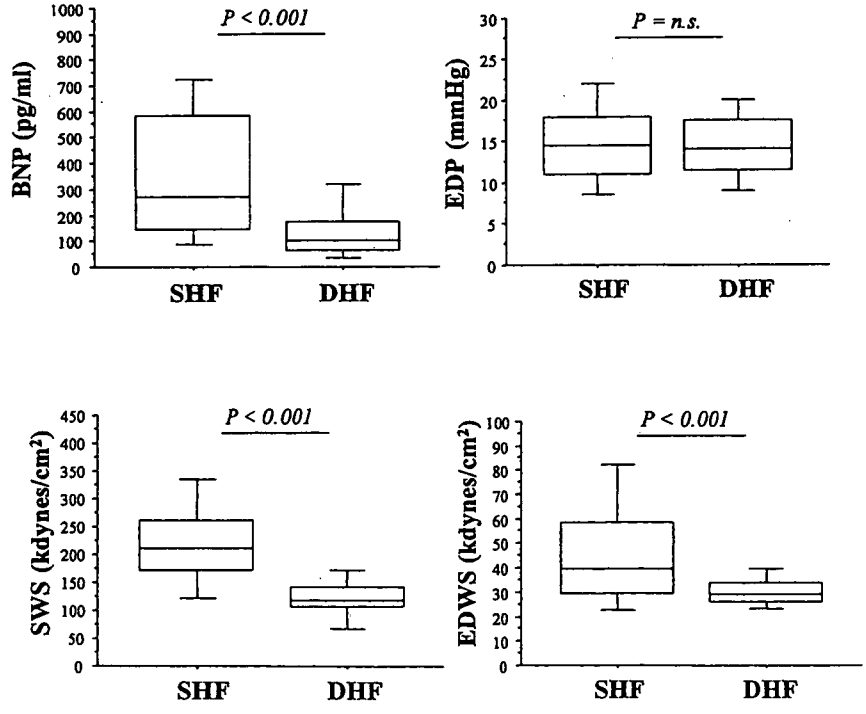

Figure 2. Differences of B-type natriuretic peptide (BNP) and left ventricular functional parameters between systolic heart failure (SHF) (n = 98) and diastolic heart failure (DHF) (n = 62). The box defines the interquartile range with the median indicated by the crossbar. The error bars indicate the 10th and 90th percentiles. EDP = end-diastolic pressure (mm Hg); EDVI = end-diastolic volume index (ml/m^2); EDWS = end-diastolic wall stress (kdynes/cm^2); SWS = end-systolic wall stress (kdynes/cm^2).

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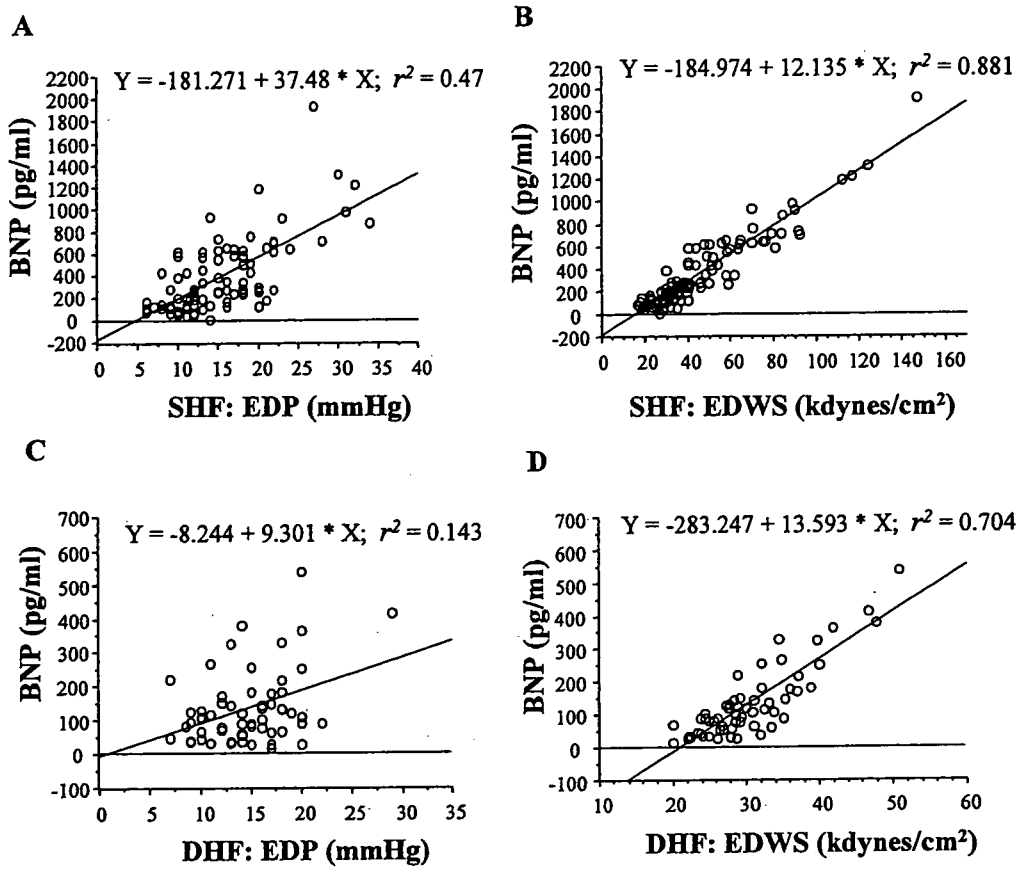

Figure 3. Correlation between B-type natriuretic peptide (BNP) and left ventricular functional parameters in 98 patients with systolic heart failure (SHF) (**A and B**) and in 62 patients with diastolic heart failure (DHF) (**C and D**); (**A and C**) end-diastolic pressure (EDP) (mm Hg) and (**B and D**) end-diastolic wall stress (EDWS) (kdynes/cm^2).

tation in addition to LV EF, age, and renal function (7). The present study demonstrates the significance of LV EDWS in the regulation of BNP in patients with HF in general. This was true not only in patients with SHF but also with DHF. Although correlation analysis suggested a relationship between other parameters of LV geometry and function including EDP and plasma BNP levels, the correlation between LV EDWS and BNP was the most robust (r^2 = 0.887). Many studies including ours have shown that BNP levels correlate well with changes in filling pressures during tailored therapy (6,17), while O'Neill et al. (18) recently reported that plasma BNP might not correlate closely with changes in intracardiac filling pressures. In any case, plasma BNP levels are not uniform across different patients with the same LVEDP (i.e., interindividual heterogeneity), and this may be because BNP is determined more by EDWS than by filling pressure. Left ventricular EDWS might account for the wide variations that they observed in patients with HF.

The present result suggests that LV EDWS may regulate BNP secretion in humans. Indeed, experiments using cultured neonatal rat ventricular cells showed that cardiac myocytes are able to respond to mechanical stretch by increasing BNP secretion and gene expression (11). Wiese et al. (19), using isolated human myocardium, have also demonstrated that, while the isometric contraction mode did not have any influence on BNP expression, diastolic overstretch increased BNP gene expression in a time-dependent manner. This implies that diastolic stretch (i.e., preload rather than afterload) seems to be the mechanical factor responsible for the induction of BNP expression and may be the reason that in the present study LV EDWS shows a better correlation with the plasma BNP levels than does LV SWS. Furthermore, in vitro studies have implicated the contributions of local paracrine and autocrine factors in the stretch-induced BNP activation (11). Local angiotensin II was shown to play a critical role in the development of stretch-induced cardiac hypertrophy and to at least partly regulate mechanical load-induced BNP expression. Recently, in addition to stimuli such as myocyte stretching and neurohumoral activation, acute myocardial hypoxia has been reported to increase cardiac BNP gene transcription and raise the plasma proBNP concentration in an animal study (20). This mechanism may explain the increase in plasma BNP in patients with acute coronary syndromes and myocardial infarction (21). In the present study, because such patients with acute ischemia were not included, the correlation between LV EDWS and plasma BNP might actually be stronger.

Myocardial wall stress is one of the primary determinants of myocardial oxygen consumption (22). Cardiac decompensation is thought to result when the feedback loop that normalizes wall stress to abnormal loading of the heart dysfunctions. The increased wall stress may act directly or indirectly via cellular mediators such as angiotensin, endothelin, inflammatory cytokines, reactive oxygen species, and matrix metalloproteinase to orchestrate a variety of molecular and cellular remodeling events determining the structural and functional properties of the myocardium and, ultimately, the rate of disease progression (23–27). Therefore, usefulness of plasma BNP levels in predicting morbidity and mortality accurately in patients with chronic HF may be explained by the relationship between the LV EDWS and BNP. Many other factors, such as age, gender, body mass, genetics, etc., are also known to affect plasma BNP levels. However, the demonstration of the link between the hemodynamics (LV EDWS) and neurohormonal factor (BNP) may support the usefulness of BNP-guided treatment of HF. Although more randomized studies are needed, pharmacotherapy guided by BNP levels is intriguing and promising (3).

There are several methods to estimate the wall stress, and we used a formula based on M-mode echocardiographic variables (16). This method may have several limitations. For example, when there is regional asynergy in LV wall motion and variation in local LV wall thickness, the estimate may not reflect the entire non-uniform LV wall stress correctly. To test this possibility, we analyzed the data of the patients without LV asynergy demonstrated by echocardiogram and LV ventriculography. We obtained an even better correlation. Interestingly, a correlation in patients with a local wall motion abnormality was still strong ($r^2 = 0.820$). There are several other limitations to our study. Echocardiography and blood sampling were typically performed the day before cardiac catheterization. This time lag could have influenced the results. A further limitation is that the study population was composed of the patients who were in stable condition and could tolerate LV cardiac catheterization; thus, patients who could not bear cardiac catheterization (e.g., patients with New York Heart Association functional class IV HF) were excluded.

In the present study, we demonstrated that plasma BNP levels strongly reflect EDWS in the LV more than any other parameter previously reported. In addition, EDWS accurately accounts for the increase in plasma BNP levels even in patients with diastolic HF. The relationship of LV EDWS to plasma BNP may give a better understanding to the interindividual heterogeneity of plasma BNP levels and its clinical utility in the diagnosis and management of HF.

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REFERENCES

1. Maisel AS, Krishnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002;347:161–7.
2. Anand IS, Fisher LD, Chiang YT, et al. Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan Heart Failure Trial (Val-HeFT). Circulation 2003;107: 1278–83.
3. Troughton RW, Frampton CM, Yandle TG, Espiner EA, Nicholls MG, Richards AM. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. Lancet 2000;355:1126–30.
4. Lubien E, DeMaria A, Krishnaswamy P, et al. Utility of B-natriuretic peptide in detecting diastolic dysfunction: comparison with Doppler velocity recordings. Circulation 2002;105:595–601.
5. Tang WH, Girod JP, Lee MJ, et al. Plasma B-type natriuretic peptide levels in ambulatory patients with established chronic symptomatic systolic heart failure. Circulation 2003;108:2964–6.
6. Maeda K, Tsutamoto T, Wada A, Hisanaga T, Kinoshita M. Plasma brain natriuretic peptide as a biochemical marker of high left ventricular end-diastolic pressure in patients with symptomatic left ventricular dysfunction. Am Heart J 1998;135:825–32.
7. Troughton RW, Prior DL, Pereira JJ, et al. Plasma B-type natriuretic peptide levels in systolic heart failure: importance of left ventricular diastolic function and right ventricular systolic function. J Am Coll Cardiol 2004;43:416–22.
8. Yamamoto K, Burnett JC Jr., Jougasaki M, et al. Superiority of brain natriuretic peptide as a hormonal marker of ventricular systolic and diastolic dysfunction and ventricular hypertrophy. Hypertension 1996; 28:988–94.
9. Yasue H, Yoshimura M, Sumida H, et al. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. Circulation 1994;90:195–203.
10. Liang F, Gardner DG. Mechanical strain activates BNP gene transcription through a p38/NF-kappaB-dependent mechanism. J Clin Invest 1999;104:1603–12.
11. Tokola H, Hautala N, Marttila M, et al. Mechanical load-induced alterations in B-type natriuretic peptide gene expression. Can J Physiol Pharmacol 2001;79:646–53.
12. Ikeda T, Matsuda K, Itoh H, et al. Plasma levels of brain and atrial natriuretic peptides elevate in proportion to left ventricular end-systolic wall stress in patients with aortic stenosis. Am Heart J 1997;133:307–14.
13. Vanderheyden M, Goethals M, Verstreken S, et al. Wall stress modulates brain natriuretic peptide production in pressure overload cardiomyopathy. J Am Coll Cardiol 2004;44:2349–54.
14. Schiller NB, Shah PM, Crawford M, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. J Am Soc Echocardiogr 1989;2:358–67.
15. Irvine T, Li XK, Sahn DJ, Kenny A. Assessment of mitral regurgitation. Heart 2002;88 Suppl 4:IV11–9.
16. Douglas PS, Reichek N, Plappert T, Muhammad A, St John Sutton MG. Comparison of echocardiographic methods for assessment of left ventricular shortening and wall stress. J Am Coll Cardiol 1987;9:945–51.
17. Kazanegra R, Cheng V, Garcia A, et al. A rapid test for B-type natriuretic peptide correlates with falling wedge pressures in patients treated for decompensated heart failure: a pilot study. J Card Fail 2001;7:21–9.
18. O'Neill JO, Bott-Silverman CE, McRae AT 3rd, et al. B-type natriuretic peptide levels are not a surrogate marker for invasive hemodynamics during management of patients with severe heart failure. Am Heart J 2005;149:363–9.
19. Wiese S, Breyer T, Dragu A, et al. Gene expression of brain natriuretic peptide in isolated atrial and ventricular human myocardium: influence of angiotensin II and diastolic fiber length. Circulation 2000;102: 3074–9.
20. Goetze JP, Gore A, Moller CH, Steinbruchel DA, Rehfeld JF,