

# Effect of Spironolactone on Plasma Brain Natriuretic Peptide and Left Ventricular Remodeling in Patients With Congestive Heart Failure

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<b>OBJECTIVES</b>	We sought to evaluate the effects of spironolactone on neurohumoral factors and left ventricular remodeling in patients with congestive heart failure (CHF).
<b>BACKGROUND</b>	Aldosterone (ALD) promotes collagen synthesis and structural remodeling of the heart. Spironolactone, an ALD receptor antagonist, is reported to reduce mortality in patients with CHF, but its influence on left ventricular remodeling has not been clarified.
<b>METHODS</b>	Thirty-seven patients with mild-to-moderate nonischemic CHF were randomly divided into two groups that received treatment with spironolactone (n = 20) or placebo (n = 17). We measured left ventricular volume and mass before treatment and after four months of treatment. We also measured the plasma levels of neurohumoral factors, such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), as well as plasma procollagen type III aminoterminal peptide (PIIINP), a marker of myocardial fibrosis.
<b>RESULTS</b>	Left ventricular volume and mass were significantly decreased and ejection fraction was significantly increased in the spironolactone group, while there were no changes in the placebo group. Plasma levels of ANP, BNP and PIIINP were significantly decreased after spironolactone treatment, but were unchanged in the placebo group. There was a significant positive correlation between the changes of PIIINP and changes of the left ventricular volume index ( $r = 0.45$ , $p = 0.045$ ) as well as the left ventricular mass index ( $r = 0.65$ , $p = 0.0019$ ) with spironolactone treatment.
<b>CONCLUSIONS</b>	These findings indicate that four months of treatment with spironolactone improved the left ventricular volume and mass, as well as decreased plasma level of BNP, a biochemical marker of prognosis and/or ventricular hypertrophy, suggesting that endogenous aldosterone has an important role in the process of left ventricular remodeling in nonischemic patients with CHF. ( <i>J Am Coll Cardiol</i> 2001;37:1228-33) © 2001 by the American College of Cardiology

Despite the outstanding success achieved with angiotensin-converting enzyme (ACE) inhibitors in the treatment of congestive heart failure (CHF), the mortality rate remains relatively high; this high mortality rate is partly due to the aldosterone (ALD) escape phenomenon in patients being treated with ACE inhibitors (1-4). Recently, spironolactone was shown to reduce the mortality of patients with CHF who had already received ACE inhibitors, digitalis and diuretics. In the Randomized Aldactone Evaluation Study (RALES) (3,5), both sudden death and death from CHF were significantly reduced by spironolactone therapy. However, despite the great impact of these findings, the mechanism by which spironolactone improves mortality remains to be fully elucidated.

Aldosterone has both myocardial and renal effects that may have profound implications for left ventricular remodeling (6). A significant association has been found between plasma ALD and left ventricular mass in patients with hypertension as well as in a population-based sample,

suggesting that ALD may be important in the modulation of cardiac structure (7-9). Recently, it was reported that the mineralocorticoid receptor, which mediates the action of ALD, is expressed in cardiomyocytes, endothelial cells and fibroblasts in the human heart (10-12). Aldosterone is secreted from the adrenal glands, and has been shown to stimulate cardiac collagen synthesis and fibroblast proliferation via activation of local mineralocorticoid receptors or an unexplained indirect mechanism (13-16).

Plasma levels of cardiac natriuretic peptides such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are increased in patients with CHF and are useful prognostic predictors, especially BNP (17-20) which is a ventricular hormone (21). The usefulness of plasma BNP is partly due to the fact that high BNP levels are significantly correlated with hemodynamic abnormalities of the left ventricular ejection fraction (LVEF) and left ventricular end-diastolic pressure (21,22). Treatment of CHF based on the plasma aminoterminal BNP level was recently reported to reduce cardiovascular events (23).

In the present study, we evaluated left ventricular volume and mass, as well as plasma levels of neurohumoral factors, such as ANP and BNP, before and after four months of treatment with spironolactone or placebo.

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#### Abbreviations and Acronyms

ACE	=	angiotensin-converting enzyme
ANP	=	atrial natriuretic peptide
ALD	=	aldosterone
BNP	=	brain natriuretic peptide
CHF	=	congestive heart failure
EDTA	=	ethylenediaminetetraacetic acid
LVEF	=	left ventricular ejection fraction
NYHA	=	New York Heart Association
PARC	=	plasma active renin concentration
PIIINP	=	procollagen type III aminoterminal peptide
RALES	=	Randomized Aldactone Evaluation Study

## METHODS

**Patients.** We studied 37 patients with stable symptomatic CHF (New York Heart Association [NYHA] functional class II or III) and LVEF <45%. There were 29 men and eight women (mean age, 64 years). The cause of heart failure was dilated cardiomyopathy in 28 patients, hypertensive heart disease in six patients and valvular heart disease in three patients. Patients were excluded if they had coronary artery disease, renal failure, hyperkalemia or liver dysfunction. Informed consent was obtained from all patients before participation in the study, and the protocol was approved by the Human Investigations Committee of our institution. Twenty-six patients were in NYHA functional class II and 11 patients were in class III. On enrollment in the study, 24 patients were being treated with diuretics, 27 with ACE inhibitors, 27 with digitalis, 14 with beta-blockers, and three with angiotensin II type-1 receptor blockers. Most of these drugs had been administered for >3 months.

**Study protocol.** This was a prospective randomized study. All of the patients were in a stable condition for at least three months before enrollment. The 37 patients with mild-to-moderate symptomatic left ventricular dysfunction were randomly divided into two groups that received treatment with spironolactone (n = 20) or placebo (n = 17). The dose of spironolactone was set at 25 mg once daily.

To measure the plasma levels of neurohumoral factors and procollagen type III aminoterminal peptide (PIIINP), blood samples were collected from an antecubital vein following supine rest for at least 30 min before and after four months of treatment with spironolactone or placebo. The samples were collected 3 h after the morning dose of medications, such as digitalis and diuretics, but before the administration of spironolactone or placebo. When four months of treatment had been completed, blood samples were also collected 3 h after the morning dose of other medications, including digitalis and diuretics, but without spironolactone or placebo being administered. M-mode echocardiography was also performed with two-dimensional monitoring using a Sonolayer phased-array sector scanner (model SSH-160A, Toshiba Co., Tokyo, Japan) in a blinded fashion before and after four months of treatment

with spironolactone or placebo. Left ventricular volumes were calculated using Teichholtz's formula, and the LVEF was determined. Left ventricular mass was calculated using previously reported method (24).

**Measurement of neurohumoral factors and PIIINP.** Blood for measurement of the plasma levels of ANP, BNP and endothelin-1 was transferred to a chilled tube containing ethylenediaminetetraacetic acid (EDTA) (1 mg/ml) and aprotinin (500 kallikrein inactivator U/ml), and then was centrifuged at 3,000 rpm for 15 min at 4°C. Thus, the plasma obtained was stored at -30°C until assay. Plasma ANP and BNP concentrations were measured with a specific immunoradiometric assay for alpha-human ANP and human BNP, respectively, using a commercial kit (Shionogi, Osaka, Japan), as previously reported (19). The plasma endothelin-1 level was determined using an antibody against synthetic endothelin-1 (Peninsula Laboratories, Inc., Belmont, California) and <sup>125</sup>I-labeled endothelin-1 (Amersham Japan, Tokyo, Japan), as previously reported (25).

Blood for measurement of the plasma levels of norepinephrine, plasma active renin concentration (PARC) and ALD was transferred to a chilled tube containing EDTA (1 mg/ml) and centrifuged at 3,000 rpm for 15 min at 4°C, after which the plasma thus obtained was stored at -30°C until assay. The plasma norepinephrine concentration was measured by high performance liquid chromatography, while PARC and ALD levels were measured using commercial radioimmunoassay kits. Plasma PIIINP levels were measured with a specific immunoradiometric assay using a commercial kit (CIS Bio International, Nagoya, Japan).

**Statistical analysis.** All results are expressed as the mean ± SEM. Univariate analysis was performed using Student *t* test. Categorical data were compared against a chi-squared distribution. Linear regression analysis was used to determine the relationship between continuous variables. A *p* value <0.05 was regarded as significant.

## RESULTS

**Clinical characteristics.** There were no differences in age, gender, NYHA functional class, etiology of heart failure, LVEF or medications on entry into the study between the spironolactone group and the placebo group (Table 1).

**Comparison of hemodynamics and neurohumoral factors before and after treatment.** There were no differences in pretreatment heart rate, mean blood pressure, LVEF, left ventricular volume index, left ventricular mass index and plasma level of PIIINP between the spironolactone group and the placebo group, and also no differences of pretreatment neurohumoral factors, such as ANP, BNP, endothelin-1, norepinephrine, PARC and ALD (Table 2). In the placebo group, there were no significant changes in these variables after four months. In the spironolactone group, there were no significant changes in heart rate, mean blood pressure and body weight, but there was improvement

**Table 1.** Clinical Characteristics of Patients in the Spirolactone and Placebo Groups

	Spirolactone (n = 20)	Placebo (n = 17)	p Value
Age (yr)	62.7 ± 2.8	65.0 ± 3.4	NS
Gender (male/female)	15/5	13/4	NS
Etiology of left ventricular dysfunction			
Dilated cardiomyopathy	15	13	NS
Hypertensive heart disease	4	2	NS
Valvular heart disease	1	2	NS
NYHA functional class			
II	14	8	NS
III	6	3	NS
Left ventricular ejection fraction (%)	32.2 ± 2.2	36.6 ± 1.6	NS
Treatment			
Furosemides	12	12	NS
Digitalis	12	15	NS
ACE inhibitors	14	13	NS
Beta-blockers	9	5	NS

ACE = angiotensin-converting enzyme; NS = not significant; NYHA = New York Heart Association.

of NYHA functional class ( $2.3 \pm 0.1$  vs.  $1.9 \pm 0.1$ ,  $p = 0.002$ ) after four months of treatment. There was a significant difference in changes of the left ventricular end-diastolic and end-systolic volume index and the left ventricular mass index between the two groups (Fig. 1). There was also a significant difference in changes in plasma level of ALD, BNP and PIIINP between the two groups (Fig. 2). **Relationship between left ventricular volume index, left ventricular mass index and PIIINP before and after spirolactone treatment.** There was significant positive correlation between changes of the plasma PIIINP level and changes of the left ventricular mass index ( $r = 0.65$ ,  $p = 0.0019$ ) (Fig. 3) or the left ventricular volume index ( $r = 0.45$ ,  $p = 0.045$ ) with spirolactone treatment. In contrast, there was no relationship between these parameters in the placebo group.

**DISCUSSION**

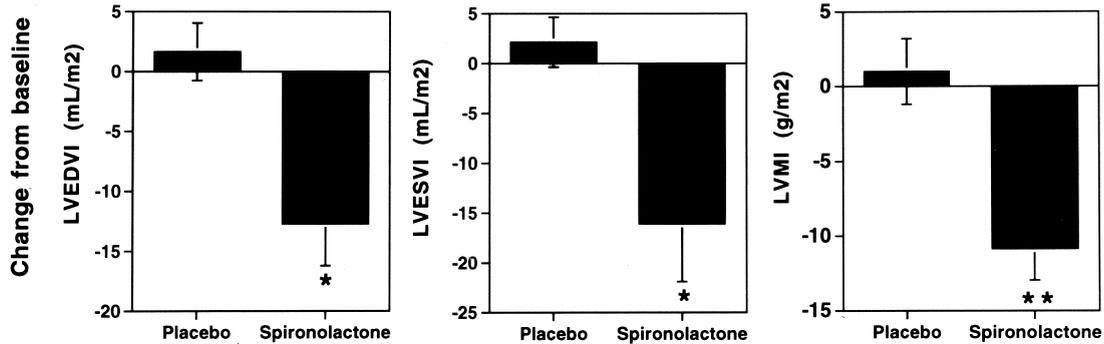
We recently reported that the heart is a target organ for ALD, and that spirolactone (a mineralocorticoid receptor antagonist) inhibits the ALD extraction through the heart in patients with CHF (26). We also found a positive correlation between the transcardiac ALD extraction and the plasma level of PIIINP (26), a possible biochemical marker of myocardial fibrosis (27,28), suggesting that the increase in ALD extraction by the failing heart might stimulate myocardial collagen turnover, as occurs in vitro (29). However, to our knowledge, serial measurements of neurohumoral factors as well as the left ventricular volume and mass have never been done before and after long-term treatment with spirolactone. The present study demonstrated for the first time that four months of treatment with spirolactone could improve left ventricular volume and mass index, as well as reduce plasma levels of BNP and PIIINP in patients with nonischemic CHF. The reduction of left ventricular volume and mass may have been due to blocking the mineralocorticoid receptor, which is known to be expressed in the human heart (14,15), since blood pressure and body weight did not change after spirolactone therapy.

Plasma ANP and BNP are useful prognostic predictors in patients with CHF, especially the level of BNP (17-20) since it is a ventricular hormone (21). The plasma BNP level was reported to be correlated with hemodynamic abnormalities of the LVEF and left ventricular end-diastolic pressure (21,22) as well as left ventricular mass (30). Therefore, the decrease of plasma BNP after treatment with spirolactone was due to the decreased left ventricular filling pressure, improvement of left ventricular remodeling or both. The decrease of BNP with spirolactone therapy may have reflected improvement of left ventricular diastolic function secondary to the effects of this drug on cardiac hypertrophy

**Table 2.** Comparison of Hemodynamics and Neurohumoral Factors Before and After Treatment in 37 Patients With Congestive Heart Failure Divided Into Two Groups: Spirolactone or Placebo

	Placebo (n = 17)		Spirolactone (n = 20)	
	Baseline	4 months	Baseline	4 months
HR (beats/min)	66.4 ± 1.9	64.8 ± 1.8	72.3 ± 3.6	70.6 ± 3.0
MBP (mm Hg)	90 ± 2.4	90.5 ± 2.8	88 ± 2.5	85.5 ± 2.4
LVEF (%)	36.6 ± 1.6	36.3 ± 1.7	32.2 ± 2.2	35 ± 1.9*
LVEDVI (ml/m <sup>2</sup> )	169 ± 15	170 ± 17	192 ± 11	178 ± 10*
LVM index (g/m <sup>2</sup> )	150 ± 10	151 ± 11	158 ± 7.8	147 ± 7.5*
PIIINP (U/ml)	0.57 ± 0.04	0.57 ± 0.05	0.64 ± 0.04	0.48 ± 0.03**
ANP (pg/ml)	67 ± 23	65 ± 23	70.9 ± 25.5	43.2 ± 17*
BNP (pg/ml)	195 ± 59	172 ± 50	200 ± 66	89.7 ± 27**
PARC (pg/ml)	234 ± 96	260 ± 66	93 ± 26	141 ± 56*
ALD (pg/ml)	88 ± 14	78 ± 8.5	61 ± 8	106 ± 16*
Norepinephrine (pg/ml)	443 ± 47	407 ± 37	382 ± 41	409 ± 43
Endothelin-1 (pg/ml)	2.9 ± 0.2	2.8 ± 0.2	2.8 ± 0.2	2.5 ± 0.2

\* $p < 0.05$ ; \*\* $p < 0.01$  versus baseline value of the spirolactone group.  
 ALD = aldosterone; ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; HR = heart rate; LVEDVI = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVM = left ventricular mass; MBP = mean arterial blood pressure; PARC = plasma active renin concentration; PIIINP = procollagen type III aminoterminal peptide.



**Figure 1.** Absolute changes from the baseline in left ventricular end-diastolic volume index (LVEDVI), left ventricular end-systolic volume index (LVESVI) and left ventricular mass index (LVMI) before and after treatment with placebo or spironolactone for four months in patients with mild-to-moderate congestive heart failure. \* $p < 0.05$ ; \*\* $p < 0.01$  versus placebo.

and fibrosis. Treatment of CHF guided by the plasma aminoterminal BNP level has been reported to reduce cardiovascular events (23), so the decrease of BNP may be associated with a beneficial outcome as was the case in the RALES study (5).

The plasma PIIINP level may be a biochemical marker of myocardial fibrosis and/or left ventricular remodeling in patients with CHF (26-28). In our previous study (26), there was a positive correlation between the plasma PIIINP level and the left ventricular end-diastolic volume index and between the transcardiac ALD extraction and the plasma PIIINP level, suggesting an interaction between ALD extraction and left ventricular remodeling in patients with CHF. In the present study, plasma PIIINP was significantly decreased after spironolactone therapy, which was consistent with the previous study (31), and there was a significant positive correlation between the changes of PIIINP and the changes of left ventricular volume and mass with spironolactone treatment. Although the cause and effect of the relationship between the changes in PIIINP and the changes in left ventricular volume and mass remain unknown, the present study suggests that PIIINP is a useful marker of the effect of spironolactone on left ventricular remodeling.

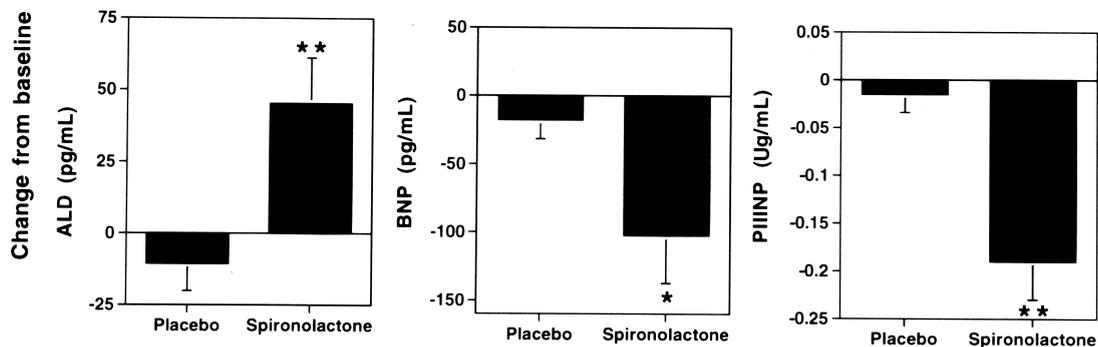
An increase of PARC and ALD has been observed with spironolactone therapy, suggesting blockade of ALD recep-

tors and loss of negative feedback inhibition (32). In the present study, the plasma levels of norepinephrine and endothelin-1 were not changed after spironolactone therapy, but we cannot deny the possibility that spironolactone may increase cardiac norepinephrine uptake and improve cardiac sympathetic activity in patients with CHF (32).

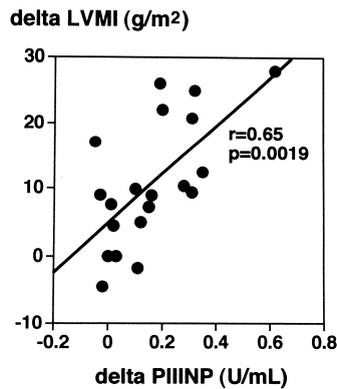
**Study limitations.** In the present study, patients with CHF secondary to coronary artery disease were excluded, since it is difficult to evaluate left ventricular volume and mass in such patients. Therefore, further studies are needed to assess the effects of spironolactone in patients with ischemic cardiomyopathy. In addition, the small number of patients with nonischemic CHF was also a limitation of the present study. The present study employed a fixed spironolactone dose of 25 mg/d, therefore, the dose-response effect of spironolactone on left ventricular remodeling and BNP must also be evaluated.

## CONCLUSIONS

Plasma levels of ANP, BNP and PIIINP were significantly decreased after four months of spironolactone treatment, but did not change in the placebo group. There was a significant positive correlation between the changes of PIIINP and the changes of left ventricular volume and mass with spironolactone therapy. These findings indicate that



**Figure 2.** Absolute changes from the baseline in plasma levels of aldosterone (ALD), brain natriuretic peptide (BNP), and procollagen type III aminoterminal peptide (PIIINP) before and after treatment with placebo or spironolactone for four months in patients with mild-to-moderate congestive heart failure. \* $p < 0.05$ ; \*\* $p < 0.01$  versus placebo.



**Figure 3.** Correlation between the changes of procollagen type III aminoterminal peptide (PIIINP) and left ventricular mass index (LVMI) after four months of spironolactone treatment in 20 patients with mild-to-moderate congestive heart failure. Delta PIIINP = pretreatment value of PIIINP - the value of PIIINP after four months, delta LVMI = pretreatment value of LVMI - the value of LVMI after four months.

long-term spironolactone treatment can improve left ventricular remodeling and decrease the plasma level of BNP, a biochemical marker of prognosis and/or ventricular hypertrophy, in patients with nonischemic CHF.

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