

## Abnormalities of sodium handling and of cardiovascular adaptations during high salt diet in patients with mild heart failure.

M Volpe, C Tritto, N DeLuca, S Rubattu, M A Rao, F Lamenza, A Mirante, I Enea, V Rendina and A F Mele

*Circulation*. 1993;88:1620-1627

doi: 10.1161/01.CIR.88.4.1620

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 1993 American Heart Association, Inc. All rights reserved.

Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circ.ahajournals.org/content/88/4/1620>

**Permissions:** Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

**Reprints:** Information about reprints can be found online at:  
<http://www.lww.com/reprints>

**Subscriptions:** Information about subscribing to *Circulation* is online at:  
<http://circ.ahajournals.org/subscriptions/>

## Abnormalities of Sodium Handling and of Cardiovascular Adaptations During High Salt Diet in Patients With Mild Heart Failure

Massimo Volpe, MD; Cristina Tritto, MD; Nicola DeLuca, MD; Speranza Rubattu, MD;  
 Maria Assunta Elena Rao, MD; Fausto Lamenza, MD; Angelina Mirante, MD;  
 Iolanda Enea, MD; Virgilio Rendina, MD; Alessandro F. Mele, MD;  
 Bruno Trimarco, MD; Mario Condorelli, MD

**Background.** Sodium retention and hormonal activation are fundamental hallmarks in congestive heart failure. The present study was designed to assess the ability of patients with asymptomatic to mildly symptomatic heart failure and no signs or symptoms of congestion to excrete ingested sodium and to identify possible early abnormalities of hormonal and hemodynamic mechanisms related to sodium handling.

**Methods and Results.** The effects of a high salt diet (250 mEq/day for 6 days) on hemodynamics, salt-regulating hormones, and renal excretory response were investigated in a balanced study in 12 untreated patients with idiopathic or ischemic dilated cardiomyopathy and mild heart failure (NYHA class I-II, ejection fraction <50%) (HF) and in 12 normal subjects, who had been previously maintained a 100 mEq/day NaCl diet. In normal subjects, high salt diet was associated with significant increases of echocardiographically measured left ventricular end-diastolic volume, ejection fraction, and stroke volume (all  $P < .001$ ) and with a reduction of total peripheral resistance ( $P < .001$ ). In addition, plasma atrial natriuretic factor (ANF) levels increased ( $P < .05$ ), and plasma renin activity and aldosterone concentrations fell (both  $P < .001$ ) in normals in response to salt excess. In HF patients, both left ventricular end-diastolic and end-systolic volumes increased in response to high salt diet, whereas ejection fraction and stroke volume failed to increase, and total peripheral resistance did not change during high salt diet. In addition, plasma ANF levels did not rise in HF in response to salt loading, whereas plasma renin activity and aldosterone concentrations were as much suppressed as in normals. Although urinary sodium excretions were not significantly different in the two groups, there was a small but systematic reduction of daily sodium excretion in HF, which resulted in a significantly higher cumulative sodium balance in HF than in normals during the high salt diet period ( $P < .001$ ).

**Conclusions.** These results show a reduced ability to excrete a sodium load and early abnormalities of cardiac and hemodynamic adaptations to salt excess in patients with mild heart failure and no signs or symptoms of congestion. (*Circulation*. 1993;88[part 1]:1620-1627.)

**KEY WORDS** • heart failure • sodium • diet • cardiomyopathies • natriuretic factors • kidney • renin

As cardiac performance declines in patients with chronic congestive heart failure (HF), various neurohormonal systems are progressively stimulated<sup>1,2</sup> in an attempt to maintain sufficient blood delivery to peripheral organs. These mechanisms may, in fact, contribute to a further progression of HF through an increase of cardiac load and avid sodium and water retention.<sup>3-6</sup>

Although the inability to excrete ingested sodium and the activation of neurohormonal mechanisms are well-characterized features of congestive HF,<sup>7-10</sup> there is limited knowledge regarding the influence of sodium

intake on hemodynamic, renal, and hormonal parameters in patients with mild HF. Despite the potential importance of a definition of sodium handling in the early or milder stages of HF, to date no balanced study has been performed in untreated patients to assess the hemodynamic, renal, and neurohormonal interactions that occur when sodium intake is manipulated. In this regard, we have recently reported that in patients with dilated cardiomyopathy and mild HF, the cardiorenal and hormonal responses to acute volume overload are impaired, even when the basal circulating levels of renin activity, aldosterone, and norepinephrine are still within the normal range.<sup>11</sup> These responses can be significantly ameliorated by cardiac unloading produced with atrial natriuretic factor (ANF),<sup>11</sup> nitroglycerin, or angiotensin converting enzyme inhibitor administration.<sup>12</sup>

The present study was undertaken to investigate whether patients with mild HF are able to excrete a sustained, moderate oral sodium load and to identify

Received March 1, 1993; revision accepted June 15, 1993.

From the First Clinica Medica, Facoltà di Medicina e Chirurgia, Università di Napoli "Federico II," Italy.

Correspondence to Dr Massimo Volpe, 1<sup>a</sup> Clinica Medica, Facoltà di Medicina, Università di Napoli "Federico II," via Pansini, 5, 80131-Napoli, Italy.

**TABLE 1. Individual Characteristics of the Patients With Mild Heart Failure**

Patient	Age/Sex	Dx	NYHA class	LVEDD, mm	LVEF, %	Vo <sub>2</sub> , mL · kg <sup>-1</sup> · min <sup>-1</sup>	ETD, min
1	31/M	ICM	I	62	41	22	12.5
2	52/M	CAD	I	56	49	18	10.2
3	58/M	CAD	II	66	25	14	9.5
4	47/M	CAD	I	61	43	15	9.9
5	58/M	CAD	II	65	29	16	10.4
6	54/M	CAD	I	59	44	20	13.2
7	28/M	ICM	I	60	48	20	12.2
8	48/M	CAD	I	70	27	18	11.6
9	49/M	ICM	I	63	42	17	8.3
10	30/F	ICM	II	64	37	16	8.5
11	50/F	ICM	II	64	35	15	10.9
12	49/F	ICM	I	62	48	16	8.4

Dx indicates diagnosis; LVEDD, echocardiographic left ventricular end-diastolic diameter; LVEF, radionuclide-assessed left ventricular ejection fraction; Vo<sub>2</sub>, peak oxygen consumption; ETD, exercise time duration; CAD, coronary artery disease; and ICM, idiopathic cardiomyopathy.

possible abnormalities of hemodynamic adaptations and of salt-regulating hormones in response to increased sodium intake in the same patients. For this purpose, the effects of a 1-week high sodium intake were assessed in a balanced study in untreated patients with mild HF and no signs or symptoms of congestion and in a control group of normal subjects.

### Methods

#### Study Subjects and Patients

The initial study population included 14 patients with chronic, stable, mild HF and no signs or symptoms of congestion. The patients were recruited in the outpatient clinic for treatment of cardiovascular diseases of our institution. Thirteen normal volunteers were also studied. All subjects gave written informed consent before participation in this study, which was approved by the ethical committee of this institution.

Normal status was established by history, physical examination, and laboratory analyses, which included a blood count, serum glucose and cholesterol concentrations, indexes of renal and hepatic function, an ECG, a chest radiograph, and M- and B-mode echocardiograms. The normal group comprised 10 male and 3 female subjects aged 38±4 years (range, 22 to 62 years).

The patients with HF included 11 male and 3 female subjects, aged 46±3 years (range, 28 to 58 years). The cause of HF was idiopathic dilated cardiomyopathy in 6 and coronary artery disease in 8 subjects. Patients were considered to have an idiopathic dilated cardiomyopathy when no obvious underlying cause of HF could be discovered during routine clinical evaluation. The diagnosis of coronary artery disease was based on the documentation of at least one myocardial infarction and on previous coronary angiography in 8 subjects.

Exclusion criteria included angina pectoris, myocardial infarction within the previous 3 months, hypertension, atrial fibrillation or severe ventricular arrhythmias, renal failure, recent acute cardiac decompensation as defined by the sudden onset of pulmonary congestion or peripheral edema, valvular disease or significant mitral

regurgitation, cardiothoracic anatomy not allowing satisfactory and reproducible recording of echocardiogram, or previous treatment with diuretics. Two of the patients who had satisfied the inclusion criteria (outlined below) were excluded from the study because of lack of compliance with dietary sodium manipulation. For the same reason, 1 normal subject was excluded (urinary sodium excretion levels at the end of the 100 mEq NaCl period were 233, 176, and 180 mEq/24 h, respectively).

The definition of mild HF was based on the following criteria. Patients showed no reduction or mild reduction in their functional capacity (class I or II according to the NYHA classification), and there was mild-to-moderate limitation of exercise capacity, as determined by cardiopulmonary exercise testing using a standard protocol (upright bicycling with a stepwise increase of 10 W/min). Mean exercise duration was 10.5±0.5 minutes. Peak oxygen consumption averaged 17.3±0.7 mL · kg<sup>-1</sup> · min<sup>-1</sup>. Echocardiographic end-diastolic left ventricular diameter exceeded 55 mm (mean, 62.7±1 mm), and left ventricular ejection fraction, as determined by radionuclide technique, was <50% (mean, 39.0±2.4%) on at least one measurement within 3 months before the study. The individual characteristics of the 12 patients who completed the study are presented in Table 1.

Three of the patients had received treatment with digitalis; nitrates had been used in 5 patients before the study, and 5 had been treated with angiotensin converting enzyme inhibitors.

#### Experimental Protocol

All drug therapy was discontinued at least 2 weeks before the beginning of the study. When subjects were treated with angiotensin converting enzyme inhibitors, 3 weeks of washout were required. Alcohol, caffeine, cigarettes, and physical exercise were all prohibited within 48 hours of the beginning of the study. Patients were admitted to the metabolic ward, where accurate assessment of daily weights (in the morning before breakfast), total intake and output, and the timing and

completion of all 24-hour urine collections were controlled by research personnel. An accurate daily record was kept of fluid, sodium, potassium, and caloric intake. All subjects received a daily diet containing 100 mEq sodium, 50 mEq potassium, 65 g protein, 50 g fat, 270 g carbohydrate, and 100 mg phosphorus in the form of pasta, meat, eggs, bread, vegetables, and fruit. Personal food preferences were allowed as much as possible, and all meals were prepared by the institution kitchen. Water intake was kept between 1500 and 1800 mL/day. Urinary volume and electrolyte excretion were measured daily throughout the diet.

After 5 days on this diet, blood samples were taken for biochemical and hormonal measurements, and non-invasive assessment of hemodynamic parameters was performed. These measurements were taken after the patients had been sitting for at least 60 minutes in a comfortable chair. Following baseline measurements, a daily sodium supplement of 150 mEq (three doses of 50 mEq crystalline sodium chloride, each wrapped in wafers administered during the three meals) was added to the diet for 6 days. Urinary volume and electrolyte excretion were measured daily, while hemodynamic observations were obtained also on days 3 and 6 of the high salt intake regimen, and blood for biochemical and hormonal measurements was collected daily on days 3 through 6 of this experimental period. Again, all measurements were obtained with the subjects in the sitting position.

Daily 24-hour urine collections were obtained beginning at 8:00 AM and ending at 8:00 AM the following morning. To ensure that sodium balance was being achieved, urine collections were promptly analyzed for volume and electrolyte excretion, and creatinine clearance was calculated. The achievement of sodium balance was demonstrated by the lack of significant differences in sodium excretion over the last 3 days of the moderate sodium diet. For analysis in this study, the 24-hour urine collection that ended on the morning of the hemodynamic study (the last day of the 100 mEq sodium diet) was used as a baseline.

### Measurements

Hemodynamic studies were performed at 9:00 AM following overnight fast in all patients. The temperature (22°C) and the lights of the study room were maintained at constant levels. Arterial blood pressure was measured by standard sphygmomanometric technique, following the recommendations of the American Heart Association<sup>13</sup> at 10-minute intervals for five times, and the average of the last three measurements was used for the analysis. Heart rate was continuously monitored by ECG lead II, and the values corresponding to arterial pressure measurements were used for the analysis. Forearm blood flow and calculated vascular resistance were measured by strain-gauge plethysmography. M- and B-mode echocardiograms were also recorded for measurements of ventricular dimensions, calculation of left ventricular ejection fraction, and estimation of stroke volume and the derived parameters.

Biochemical and hormonal tests included serum electrolytes, blood urea nitrogen and creatinine, peripheral hematocrit, plasma ANF levels, plasma renin activity (PRA) and aldosterone concentrations, and venous plasma norepinephrine concentrations (only at baseline).

### Laboratory Methods

Blood samples for plasma ANF and norepinephrine levels were collected on ice and spun immediately (within 10 minutes); blood samples for measurement of PRA and aldosterone concentrations were collected at room temperature. The plasma was then separated and frozen until the time of assay, which did not exceed 4 weeks.

PRA was measured by enzymatic assay,<sup>14</sup> in which plasmas were incubated at 37°C for 3 hours in the presence of endogenous angiotensinogen and converting enzyme and angiotensinase inhibitors (phenylmethylsulfonyl fluoride and EDTA). pH was adjusted at 5.7 with maleic acid. The angiotensin I formed was quantitated by radioimmunoassay, and results were expressed as the hourly rate of angiotensin I generation ( $\text{ng} \cdot \text{mL}^{-1} \cdot \text{h}^{-1}$ ) (sensitivity, 50 pg/tube angiotensin I, intra-assay and interassay variability coefficients, 6% and 10%, respectively). Plasma immunoreactive ANF levels were determined by radioimmunoassay as previously described by our laboratory,<sup>15</sup> by using rabbit antiserum (RAS 8798, Peninsula Lab, Europe), iodinated human ANF-(99-126) (2000 Ci/mmol, Amersham Berks, UK), and  $\alpha$ -human ANF-(99-126) (Novabiochem, Switzerland) as a standard. ANF was extracted from plasma with Sep-Pak C<sub>18</sub> cartridges. The recoveries determined on each plasma sample by adding to it a minimal amount of radiolabeled ANF ranged from 75% to 90%. Intra-assay and interassay variation coefficients were 6.8% and 10.1%, respectively. The radioimmunoassay sensitivity was 1 fmol per tube. Plasma aldosterone concentrations were estimated by a radioimmunoassay technique using a commercial kit (DPC, Los Angeles, Calif). Plasma norepinephrine assay was performed with reverse-phase high-performance liquid chromatography with electrochemical detection, after extraction and concentration by adsorption onto activated alumina.<sup>16</sup> Potassium and sodium levels in urine were measured by ion-selective electrodes (Beckman E2A Na/K system, Arlington Heights, Ill).

### Echocardiographic Measurements

Wide-angle, two-dimensional echoes were recorded using a phased-array sector scanner (77020 AC, Hewlett-Packard Co, Andover, Mass). All studies were videotaped on 3/4-in. videocassette recorders equipped with a back-spacer search module, which allows frame-by-frame bidirectional playback. The video frame rate of the system is approximately 60 frames per second.

All patients were studied in the sitting position using multiple views through the apical window. Two views were selected for measurements: apical-four chamber and apical two-chamber. The left ventricular long axis ( $L_{\text{max}}$ ) was measured at end diastole as the longest major axis in either of the two apical views. The measurements of  $L_{\text{max}}$  were rounded off to the closest whole number to ensure reproducibility. Left ventricular end-diastolic area (EDA) was measured using the largest of all the left ventricular minor axes measured. Left ventricular end-diastolic volume (EDV) was calculated according to the single plane ellipse method as<sup>17</sup>:

$$\text{EDV (mL)} = \frac{8 \text{ EDA}^2}{3 \pi \cdot L_{\text{Max}}}$$



The same measurements were undertaken in end systole to calculate end-systolic volume. Ejection fraction was measured using the averages of all the end-diastolic and end-systolic volumes.<sup>17</sup> Stroke volume was derived as the difference between end-diastolic and end-systolic volumes, and cardiac output and total peripheral resistance were estimated by using standard formulas.

All studies were performed by the same investigator and read independently by two experts unaware of the protocol. The readings obtained showed correlations for both  $L_{\max}$  ( $r=.97$ ,  $P<.001$ ) and EDA ( $r=.96$ ,  $P<.001$ ). Excellent correlations were also obtained for the measurements of end-diastolic volume ( $r=.97$ ,  $P<.001$ ) and end-systolic volume ( $r=.96$ ,  $P<.001$ ) between the two observers. The variability of separate measurements of ventricular volumes obtained in different days in the absence of any treatment or dietary changes did not exceed 3%. The echocardiographic measurements of stroke volume were significantly correlated with the measurements obtained by thermodilution technique ( $r=.80$ ,  $P<.01$ ). Radionuclide assessment of ejection fraction for classification of the patients was performed with the patient at rest in the supine position according to the methods previously reported from this laboratory.<sup>18</sup> Individual echocardiographic measurements of ejection fraction were significantly correlated with the corresponding radionuclide ejection fraction values obtained in our patients ( $r=.75$ ,  $P<.01$ ).

#### Measurements of Forearm Blood Flow

Forearm blood flow was measured with a mercury-in-Silastic strain-gauge plethysmograph using a venous occlusion technique as previously described.<sup>19</sup> The strain gauge was placed approximately 5 cm below the antecubital crease. Forearm blood flow ( $\text{mL} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ ) was calculated from the rate of increase in forearm volume, while venous return from the forearm was prevented by inflating the cuff at the upper arm. The pressure in the venous occlusion or congesting cuff at the upper arm was 40 mm Hg. Circulation of the hand was arrested by inflating a cuff around the wrist. The wrist cuff was inflated before determining forearm blood flow and continuously throughout the measurements. Blood pressure was determined with the mercury sphygmomanometric method in the same arm. Forearm vascular resistances ( $\text{mm Hg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \cdot 100 \text{ g}$ ) were calculated by dividing mean blood pressure by forearm blood flow. Mean blood pressure was calculated by adding to diastolic blood pressure one third of pulse pressure.

#### Statistical Analysis

Data are presented as mean $\pm$ SEM. Distribution of the data was assessed by the Bartlett test.  $\chi^2$  analysis was used for comparison of descriptive parameters. Comparisons of the basal data of the different groups were performed by unpaired  $t$  test or Wilcoxon rank test, as appropriate. One-way ANOVA for repeated measures followed by post-hoc comparisons was performed to detect changes over time within the same group. Between-groups comparisons of the responses were tested by two-way ANOVA (factoring for group and time).

**TABLE 2. Clinical and Laboratory Characteristics of the Study Groups**

	HF (n=12)	N (n=12)
Age, y	46 $\pm$ 3	38 $\pm$ 4
Sex, male/female	9/3	9/3
Weight, kg	66.2 $\pm$ 3.1	69.1 $\pm$ 4.0
Blood glucose, mg/dL	100 $\pm$ 5	96 $\pm$ 3
BUN, mg/dL	35 $\pm$ 1	40 $\pm$ 3
Serum creatinine, mg/dL	1.0 $\pm$ 0.05	1.0 $\pm$ 0.04
SBP/DBP, mm Hg	115 $\pm$ 3/76 $\pm$ 3	113 $\pm$ 4/76 $\pm$ 2
HR, bpm	66 $\pm$ 2	67 $\pm$ 3
U <sub>Na</sub> V, mEq/24 h	98 $\pm$ 6	102 $\pm$ 7
U <sub>K</sub> V, mEq/24 h	40 $\pm$ 4	41 $\pm$ 4
PNE, pg/mL	408 $\pm$ 39	360 $\pm$ 29

HF indicates patients with mild heart failure; N, normal subjects; BUN, blood urea nitrogen; SBP/DBP, supine systolic/diastolic blood pressure; HR, heart rate; U<sub>Na</sub>V, urinary sodium excretion rate; U<sub>K</sub>V, urinary potassium excretion rate; and PNE, plasma venous norepinephrine concentration in the sitting position on a moderately low sodium diet. No significant differences were found between the groups. Data are expressed as mean $\pm$ SEM.

## Results

### Characteristics of the Study Groups

The two study groups (normal subjects and patients with mild HF) were comparable with regard to clinical characteristics and renal function (Table 2 and Fig 1). In addition, PRA, plasma aldosterone, venous plasma norepinephrine concentrations, and urinary volume and electrolyte excretion were not different in the two groups. In contrast, plasma ANF levels were about threefold higher in HF patients than in normal controls ( $P<.05$ ).

The basal values of the hemodynamic parameters are shown in Fig 1. In HF patients, both left ventricular volumes were significantly higher ( $P<.01$ ) and ejection fraction was lower ( $P<.001$ ) than in normal subjects, whereas stroke volume and peripheral resistance were comparable in the two groups. The subjects from the two groups who showed satisfactory compliance to the diet, as defined in "Methods," were studied (normal subjects, n=12; HF patients, n=12).

### Hemodynamic Responses to High Salt Diet

Fig 1 shows that the cardiac and systemic hemodynamic responses to high salt intake were largely different in the two groups. In the control group (left panels), left ventricular end-diastolic volume increased in response to high salt diet on days 3 and 6 as compared with baseline ( $F=19.9$ ,  $P<.001$ ), whereas left ventricular end-systolic volume did not change ( $F=.44$ ). Accordingly, left ventricular ejection fraction was increased on both days 3 and 6 of the high salt diet compared with baseline ( $F=18.2$ ,  $P<.001$ ), and stroke volume was increased on days 3 and 6 compared with baseline ( $F=21.1$ ,  $P<.001$ ). Finally, calculated total peripheral resistance (TPR) was progressively reduced ( $F=16.2$ ,  $P<.001$ ) and was significantly different from baseline on days 3 and 6.

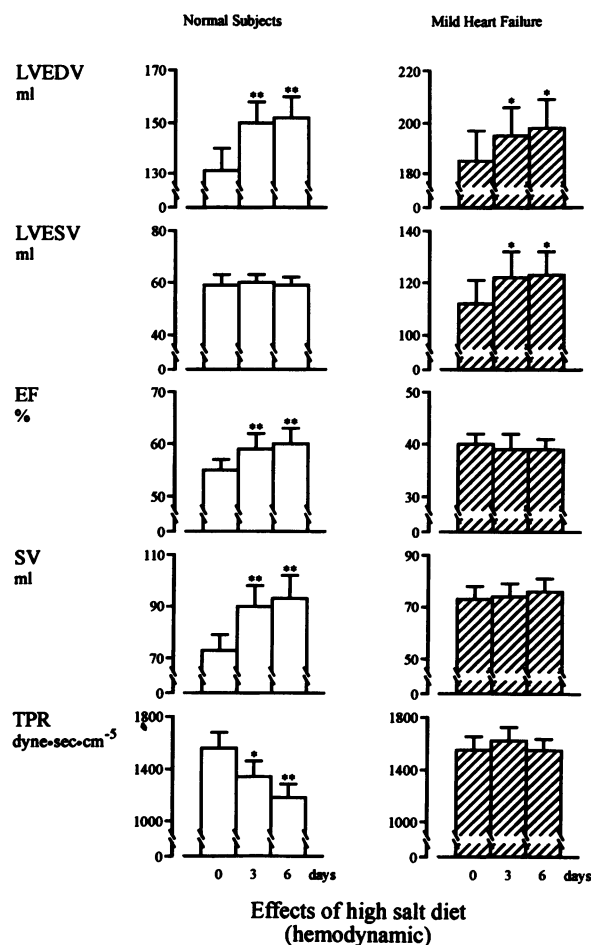


FIG 1. Bar graph of hemodynamic effects of high salt diet. LVEDV indicates left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; EF, ejection fraction; SV, stroke volume; and TPR, total peripheral resistance. \* $P < .05$  or \*\* $P < .001$ , respectively, vs baseline.

In contrast, in HF patients (Fig 1, right panels), both left ventricular end-diastolic volume ( $F=7.30$ ,  $P < .01$ ) and left ventricular end-systolic volume ( $F=8.87$ ,  $P < .001$ ) increased significantly. Left ventricular ejection fraction did not increase during high salt diet in this group ( $F=2.09$ ,  $P=NS$ ). Furthermore, stroke volume failed to increase ( $F=0.94$ ,  $P=NS$ ), and TPR remained unchanged ( $F=0.82$ ,  $P=NS$ ) in HF patients during high salt diet, in sharp contrast with the behavior of the same parameters observed in normals.

Daily measurements of heart rate did not show any significant change in either group during the study ( $F=1.5$  and  $1.0$ , in normal and HF subjects, respectively; both  $P > .05$ ). Systolic blood pressure did not change significantly in either group. Diastolic blood pressure did not change in HF patients (from  $76 \pm 3$  mm Hg at baseline to  $78 \pm 3$  mm Hg on day 6,  $F=4.9$ ,  $P=NS$ ) but showed a significant reduction in normal subjects, which was accounted for by a significant decrease on day 6 ( $65 \pm 2$  mm Hg) compared with baseline ( $76 \pm 2$  mm Hg) ( $F=11.4$ ,  $P < .01$ ). Forearm vascular resistance, estimated by strain-gauge plethysmography, showed a slight albeit not significant reduction in normals (from  $46 \pm 4$  to  $41 \pm 3$  and  $39 \pm 3$  mm Hg  $\cdot$  mL<sup>-1</sup>  $\cdot$  min<sup>-1</sup> on days 3 and

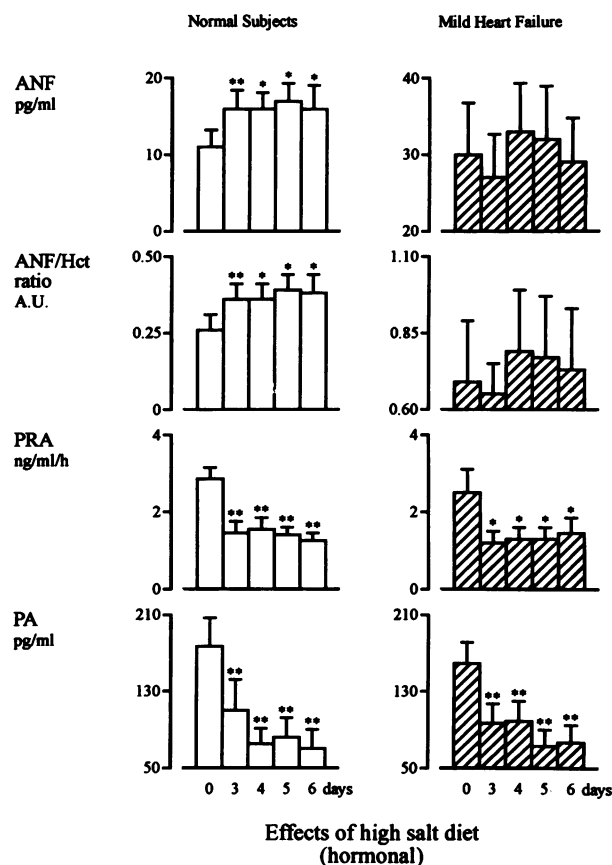


FIG 2. Bar graph of effects of high salt diet on salt-regulating hormones. ANF indicates plasma atrial natriuretic factor levels; Hct, hematocrit; PRA, plasma renin activity; and PA, plasma aldosterone. \* $P < .05$  or \*\* $P < .001$ , respectively, vs baseline.

6, respectively;  $F=3.04$ ,  $P=.07$ ) and no change in HF (from  $56 \pm 5$  to  $58 \pm 6$  and  $53 \pm 6$  mm Hg  $\cdot$  mL<sup>-1</sup>  $\cdot$  min<sup>-1</sup>,  $F=.74$ ,  $P=NS$ ).

Finally, peripheral hematocrit showed a progressive and comparable fall in the two groups (normals: from  $44 \pm 1\%$  to  $41 \pm 1\%$ ,  $F=8.65$ ,  $P < .001$ ; HF patients: from  $44 \pm 1\%$  to  $41 \pm 1\%$ ,  $F=15.4$ ,  $P < .001$ ; two-way ANOVA:  $F=0.58$ ,  $P=NS$ ).

#### Hormonal Responses to High Salt Diet

In normal subjects (left panels), high salt intake was associated with an increase of plasma ANF levels that was observed consistently in all individuals, resulting in a significant change of mean plasma ANF concentrations ( $F=4.03$ ,  $P < .05$ ) and of plasma ANF concentrations normalized by the hematocrit corresponding values ( $F=4.97$ ,  $P < .01$ ) (Fig 2). This increase of ANF became significant on day 3 and was sustained throughout the high salt diet. In the same group, high salt intake was associated with reductions of plasma renin activity (PRA) ( $F=10.7$ ,  $P < .001$ ) and aldosterone concentrations ( $F=10.6$ ,  $P < .001$ ), which were significant on day 3 and remained significantly different from baseline throughout the diet (Fig 2).

In HF patients (Fig 2, right panels), plasma ANF concentrations did not change significantly in response to the high salt regimen ( $F=1.07$ ,  $P=NS$ ). Similarly, the

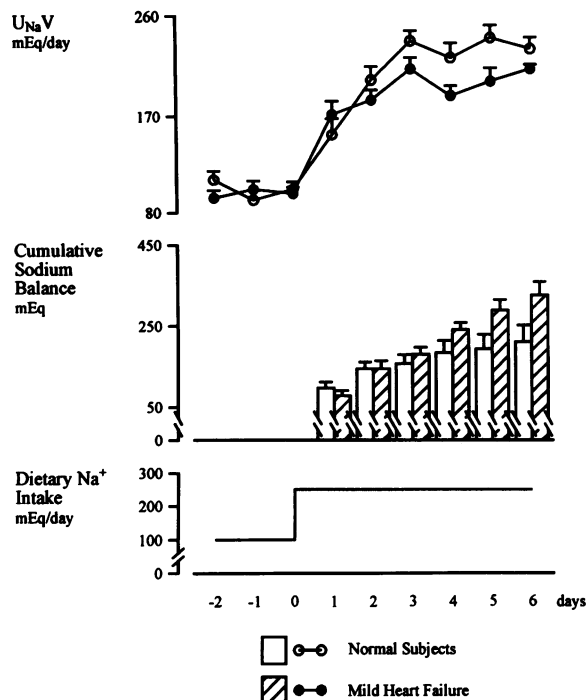


FIG 3. Sodium excretion ( $U_{Na}V$ ) and net external sodium balance when normal and dilated cardiomyopathy patients shift from 100 mEq/day to 250 mEq/day NaCl diet. The small but systemic difference in sodium excretion results in a progressive difference in cumulative sodium balance (two-way ANOVA,  $F=5.03$ ,  $P<.001$ ).

ANF-to-hematocrit ratio did not change significantly in this group ( $F=1.09$ ,  $P=NS$ ). PRA ( $P<.05$ ) and plasma aldosterone concentrations ( $P<.001$ ) were suppressed during high salt intake also in HF patients, and the degrees of suppression were not different from those observed in normal subjects (two-way ANOVA: both  $P>.05$ ).

#### Effects of Salt Loading on Urinary Electrolyte Excretion

Fig 3 shows the effects of elevated salt intake on urinary sodium excretion ( $U_{Na}V$ ) and on cumulative sodium balance in the two study groups.  $U_{Na}V$  increased significantly in both normals ( $F=24.2$ ,  $P<.001$ ) and in HF patients ( $F=14.3$ ,  $P<.001$ ), and the responses in the two groups were not significantly different ( $F=1.63$ ,  $P=NS$ ) (Fig 3). However, when cumulative sodium balance was calculated (Fig 3), a significantly more marked sodium retention was found in HF patients (two-way ANOVA:  $F=5.03$ ,  $P<.001$ ). In particular, the daily cumulative sodium balance attained in the two groups was similar in the first 3 days of high salt diet, while it diverged significantly in the second half of the diet.

Urinary volume significantly increased in normals (from  $1164\pm 143$  to  $1364\pm 135$  mL/24 h on day 6,  $F=6.5$ ,  $P<.001$ ), whereas the response in HF patients did not achieve statistical significance (from  $1218\pm 162$  to  $1376\pm 198$  mL/24 h,  $F=1.75$ ,  $P=NS$ ). However, between-groups analysis did not show significant differences.

Finally, body weight showed a slight but significant increase in HF patients (from  $66.2\pm 3$  to  $66.6\pm 3$  kg on day 6,  $F=2.7$ ,  $P<.05$ ), whereas it remained unchanged in normal subjects (from  $69.1\pm 4$  to  $69.2\pm 4$  kg,  $F=0.63$ ,  $P=NS$ ).

#### Discussion

Sodium retention and volume overload are fundamental hallmarks of the HF syndrome, and most of the symptoms and physical signs that occur in this condition result in large measure from the inability to excrete sodium and water. Therefore, the assessment of the ability to excrete ingested sodium, as well as of the circulatory and endocrine adaptations to salt intake, represents an important target in the characterization of the initial or milder stages of HF.

The present study demonstrates that hemodynamic, hormonal, and renal adaptations to salt excess are already altered in patients with mild HF, mild or no limitation of physical exercise, and no signs or symptoms of congestion. In particular, our results show that HF patients exhibited an impairment of sodium handling, as shown by the significantly higher cumulative sodium balance attained during high salt diet; these patients were unable to normally adjust cardiac performance in response to high salt intake, as indicated by the increase in left ventricular end-systolic volume as well as by the lack of increases in ejection fraction and stroke volume; HF patients also failed to lower total peripheral resistance, as observed in normals during oral salt load; and the physiological increase in plasma ANF levels in response to salt excess was absent in these patients.

Although hormonal and hemodynamic interactions with sodium retention have been extensively investigated in patients with congestive HF,<sup>7-10</sup> there are very few data concerning the beginning of the activation of neurohormonal pathways and no controlled study on the onset of sodium retention in patients with initial or milder forms of HF. A study by Kubo et al<sup>20</sup> demonstrated that renin-angiotensin and sympathetic nervous systems are not activated in the early symptomatic stages of HF. More recent data by Francis and coworkers in a substudy of the SOLVD studies<sup>21</sup> indicate that neuroendocrine activation (elevated plasma norepinephrine, ANF, and arginine-vasopressin basal concentrations) occurs in patients with moderate left ventricular dysfunction (ejection fraction  $\leq 35\%$ ) and no overt congestive HF. Finally, recent data from our laboratory<sup>11,12</sup> demonstrated that, in the basal state, patients with dilated cardiomyopathy and milder HF than those studied in the SOLVD<sup>21</sup> (mean ejection fraction was approximately 40% in our studies and 29% in the SOLVD) had elevated plasma ANF levels but normal plasma norepinephrine, renin activity, and aldosterone concentrations. The same patients, however, displayed an inadequate hemodynamic response to acute intravenous saline loading, associated with an inability to raise peripheral ANF levels and with a blunted natriuretic response.

The present study extends our previous findings<sup>11,12</sup> by showing that abnormal cardiorenal and endocrine responses are unmasked by chronic oral salt load in the same category of patients. It should be emphasized that the abnormal adaptations were produced in untreated patients with no signs or symptoms of congestion,



normal or mildly reduced tolerance to exercise and relatively preserved hemodynamic function, hormonal profile, and sodium excretory properties at baseline. Thus, our current results may have pathophysiological relevance with respect to sodium handling in early or mild HF, also in view of the moderate degree of salt excess adopted.

The echocardiographic technique used to ensure multiple, noninvasive measures of cardiac and hemodynamic variables in a prolonged dynamic protocol may result in less accurate determinations of absolute ventricular volumes in patients with dilated cardiomyopathy. Our conclusions, however, are based on repeated within-patient measurements and therefore are less affected by the limitations of the technique. In fact, the variability of ventricular volume measurements in the absence of interventions did not exceed 3% in our HF patients.

With regard to our cardiac and hemodynamic findings, in HF patients high salt intake was associated with notable shifts from the physiological adaptations observed in normals. In particular, both end-diastolic and end-systolic left ventricular volumes significantly increased during the diet, suggesting incomplete emptying of the left ventricle. Accordingly, left ventricular ejection fraction and stroke volume failed to increase in response to high salt intake. This hemodynamic pattern, and particularly the inability to increase ejection fraction, may be related to the location of these patients on the Frank-Starling curve and suggests a depressed ventricular response to increased preload. On the other hand, the lack of an appropriate reduction in cardiac afterload, which is indicated by the failure to decrease total peripheral resistance and systemic blood pressure in HF patients, may have, in turn, contributed to the compromised left ventricular dynamic response to increased preload.

An abnormal cardiac adaptation to salt loading is suggested in HF patients also by the impaired cardiac endocrine response, as they showed a complete inability to raise plasma ANF levels. In contrast, normal subjects coherently showed a sustained increase in plasma ANF levels of about 40% in response to increased sodium intake. Although the absolute ANF response to salt was relatively modest also in normal subjects, it should be pointed out that the measurements were obtained in the sitting position, which attenuates the ANF response to salt. This has been demonstrated by Hollister and coworkers<sup>22</sup> in normal subjects in which the ANF response to salt load (8 g) was about threefold smaller in the upright than in the supine position. The lack of increase in peripheral ANF levels in HF patients is most likely related to the failure of cardiac output to rise in response to salt-induced volume expansion, as supported by previous data obtained in our laboratory.<sup>11</sup>

The most important finding of our study is the observation that HF patients developed significant sodium retention during high salt diet, despite having no signs or symptoms of congestion, and more importantly, their urinary sodium excretion rates were normal on a regimen of moderately low sodium intake. In particular, when shifted from low to high sodium intake, they showed a slightly smaller sodium excretion compared with normals that occurred systematically over the time of the study and thus resulted in a significant difference

in cumulative sodium balance. Therefore, while normal subjects substantially achieved neutral sodium balance within 3 days from the beginning of the high salt diet, HF patients had not yet achieved external balance within the 6 days of the experimental period.

Our study does not permit us to define the exact mechanisms of sodium retention in HF patients since renal hemodynamics and function studies were not included in our protocol. This response, however, was not secondary to an impaired adaptation of the renin-angiotensin-aldosterone system since the responses of these hormones to high salt intake were not different in HF and in normals, thus confirming that these mechanisms are still preserved in this stage of the disease.<sup>20</sup> It is also unlikely that the systemic blood pressure response may have contributed to the delayed natriuresis in HF since blood pressure was reduced in normals but not in HF patients. It is possible that the absence of an appropriate hemodynamic response to salt excess and, particularly, of a rise in cardiac output<sup>23</sup> played a role in the blunted renal excretory response of HF patients. In fact, in chronic HF, the reduction of cardiac output results in decreased blood flow to several regional vascular beds, especially the kidney.<sup>24-26</sup>

The present study identifies a concealed predisposition to sodium retention in patients with mild HF that is unmasked during chronic moderate sodium excess. The blunted natriuretic adaptation to oral salt loading is associated with altered hemodynamic responses and failure to increase peripheral ANF levels. Altogether, our results show that high salt intake reveals an impaired ability to excrete ingested sodium and an inadequate cardiac response to increased preload in patients who are still exempt from congestion. These findings underscore a susceptibility to retain sodium and water early in the development of heart failure and may indirectly support the usefulness of sodium restriction and of early therapeutic interventions even in the milder stages of the disease.

#### Acknowledgment

Supported in part by Italian National Research Council (CNR) grant 91.00128,PF41 (targeted project Prevention and Control Disease Factors; subproject Cardiomyopathies).

#### References

1. Levine TB, Francis GS, Goldsmith SR, Simon AB, Cohn JN. Activity of the sympathetic nervous system and the renin-angiotensin system assessed by plasma hormone levels and their relationship to hemodynamic abnormalities in congestive heart failure. *Am J Cardiol.* 1982;49:1659-1666.
2. Thomas JA, Marks BH. Plasma norepinephrine in congestive heart failure. *Am J Cardiol.* 1978;41:233-243.
3. Cody RJ, Laragh JH. The role of the renin-angiotensin-aldosterone system in the pathophysiology of chronic CHF. In: Cohn JN, ed. *Drug Treatment of Heart Failure*. New York: Yorke Medical Books; 1983:35-51.
4. Francis GS, Goldsmith SR, Levine TB, Olivari MT, Cohn JN. The neurohormonal axis in CHF. *Ann Intern Med.* 1984;101:370-377.
5. Cohn JN, Mashiro I, Levine TB, Mehta J. Role of vasoconstrictor mechanisms in the control of left ventricular performance of the normal and damaged heart. *Am J Cardiol.* 1979;44:1019-1022.
6. Kubo SH. Neurohormonal activation and the response to converting enzyme inhibitors in congestive heart failure. *Circulation.* 1990;2(suppl III):III-107-III-113.
7. Braunwald E, Plauth WH Jr, Morrow AG. A method for the detection and quantification of impaired sodium excretion: results of an oral sodium tolerance test in normal subjects and in patients with heart disease. *Circulation.* 1965;32:223-231.



8. Ayers CR, Bowden RE, Schrank JP. Mechanisms of sodium retention in congestive heart failure. *Adv Exp Med Biol.* 1972;17:227-243.
9. Chonko AM, Bay WH, Stein JH, Ferris TF. The role of renin and aldosterone in salt retention of edema. *Am J Med.* 1977;63:881-889.
10. Cody RJ, Covit AB, Schaer GL, Laragh JH, Sealey JE, Feldschuh J. Sodium and water balance in chronic congestive heart failure. *J Clin Invest.* 1986;77:1441-1452.
11. Volpe M, Tritto C, DeLuca N, Mele AF, Lembo G, Rubattu S, Romano M, deCampora P, Enea I, Ricciardelli B, Trimarco B, Condorelli M. Failure of atrial natriuretic factor to increase with saline load in patients with dilated cardiomyopathy and mild heart failure. *J Clin Invest.* 1991;88:1481-1489.
12. Volpe M, Tritto C, DeLuca N, Rubattu S, Mele AF, Lembo G, Enea I, deCampora P, Rendina V, Romano M, Trimarco B, Condorelli M. Angiotensin-converting enzyme inhibition restores cardiac and hormonal responses to volume overload in patients with dilated cardiomyopathy and mild heart failure. *Circulation.* 1992;86:1800-1809.
13. Kirkendall MW, Burton MC, Epstein FM, Freis ED. Recommendations for human blood pressure determination by sphygmomanometers. *Circulation.* 1967;36:980-986.
14. Preibisz JJ, Sealey JE, Aceto RM, Laragh JH. Plasma renin activity measurements: an update. *Cardiovasc Rev Rep.* 1982;5:787-804.
15. Volpe M, Lembo G, DeLuca N, Lamenza F, Indolfi C, Condorelli GL, Trimarco B. Converting enzyme inhibition prevents the effects of atrial natriuretic factor on arterial baroreflexes. *Circulation.* 1990;82:1214-1221.
16. Trimarco B, Lembo G, Ricciardelli B, DeLuca N, Rendina V, Condorelli GL, Volpe M. Salt-induced plasticity in cardiopulmonary baroreceptor reflexes in salt-resistant hypertensive patients. *Hypertension.* 1991;18:483-493.
17. Folland ED, Parisi AF, Moghian BS, Joney DR, Feldman CL, Tow DE. Assessment of left ventricular ejection fraction and volumes by real-time, two-dimensional echocardiography. *Circulation.* 1979;60:760-771.
18. Cuocolo A, Esposito S, Acampora C, Squame G. Diagnosis of coronary disease in hypertensive patients: comparison of exercise radionuclide ventriculography and thallium-201 myocardial scintigraphy. *Radiol Med.* 1988;75:160-165.
19. Levy BI, Valladares WR, Ghaem A, Martineaud JP. Comparison of plethysmographic methods with pulsed Doppler blood flowmetry. *Am J Physiol.* 1979;6:899-903.
20. Kubo SH, Clark M, Laragh JH, Borer JS, Cody RJ. Identification of normal neurohormonal activity in mild congestive heart failure and stimulating effect of upright posture and diuretics. *Am J Cardiol.* 1987;60:1322-1328.
21. Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang C, Kubo SH, Rudin-Toretsky E, Yusuf S for the SOLVD Investigators. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure: a substudy of the Studies Of Left Ventricular Dysfunction (SOLVD). *Circulation.* 1990;82:1724-1729.
22. Hollister AS, Tanaka I, Imada T, Onrot J, Biaggioni I, Robertson D, Inagami T. Sodium loading and posture modulate human atrial natriuretic factor plasma levels. *Hypertension.* 1986;8(suppl II):II-106-II-111.
23. Cody RJ, Ljungman S, Covit AB, Kubo SH, Sealey JE, Pondolfino K, Clark M, James G, Laragh JH. Regulation of glomerular filtration rate in chronic congestive heart failure patients. *Kidney Int.* 1988;34:361-367.
24. Merrill AJ. Edema and decreased renal blood flow in patients with chronic congestive heart failure: evidence of "forward failure" as the primary cause of edema. *J Clin Invest.* 1946;25:389-400.
25. Werkö L, Varnauskas E, Eliasch H, Ek J, Bucht H, Thomasson B, Bergström J. Studies on the renal circulation and renal function in mitral valvular disease. I. Effect of exercise. *Circulation.* 1954;9:687-699.
26. Leithe ME, Margorien RD, Hermiller JB, Unverferth DV, Leier CV. Relationship between central hemodynamics and regional blood flow in normal subjects and in patients with congestive heart failure. *Circulation.* 1984;69:57-64.