OBJECTIVES
We tested the hypothesis that salt and fluid retention in heart-transplant recipients (HTRs) is caused by a failure to reflexively suppress the renin-angiotensin-aldosterone system (RAAS).

BACKGROUND
It is known that extracellular fluid volume is expanded (12% to 15%) in HTRs who develop hypertension.

METHODS
Responses to volume expansion were measured in eight HTRs (ages 57 ± 6 years) and six liver-transplant recipients (LTRs) (ages 52 ± 2 years) both before and after treatment with captopril (225 mg/day). After three days of a standardized diet, 0.154 mol/l saline was infused at 8 ml/kg/h for 4 h. Blood pressure, hormones, and renal function were monitored for 48 h. After four months, the same subjects received captopril (225 mg/day), and the protocol was repeated.

RESULTS
Before captopril, saline infusion suppressed the RAAS in LTRs but not in HTRs, resulting in elimination of 86 ± 12% versus 50 ± 11% of the sodium load by 48-h postinfusion. Blood pressure increased only in the HTRs (16 ± 5/9 ± 3 mm Hg) and remained elevated for 48 h (p ≤ 0.05). After captopril, sodium elimination was comparable in the liver (87 ± 13%) and heart groups (86 ± 12%) and blood pressure did not change in either group.

CONCLUSIONS
Heart transplant recipients have blunted diuretic and natriuretic responses to volume expansion that is mediated by their inability to suppress the RAAS. Pharmacologic suppression of the RAAS normalized defects in blood pressure and fluid homeostasis. These findings indicate that hypertension in HTRs is caused, in part, by a failure to reflexively suppress the RAAS when these patients become hypervolemic. (J Am Coll Cardiol 2003; 41:426–32) © 2003 by the American College of Cardiology Foundation

Hypertension is a frequent complication of heart transplantation with as many as 80% of heart-transplant recipients (HTRs) requiring treatment for this disorder (1–3). The underlying pathogenic mechanisms remain unclear. Post-transplant hypertension is unrelated to pre-existing hypertension and refractory to standard antihypertensive pharmacologic therapy in some HTRs (1–3). Mechanisms responsible for de novo hypertension in HTRs include vascular stiffness and endothelial dysfunction, which appear to persist indefinitely after transplantation (3), and cyclosporine-mediated side effects, including sympathoexcitation (4) and nephrotoxicity (5). However, hypertension in HTRs is more severe than in other transplant populations and reductions in cyclosporine dose, serum concentration, and duration of therapy fail to reduce the incidence of hypertension (1–3). Moreover, HTRs who never receive cyclosporine also have a high incidence of hypertension (6). Against this background, it is appropriate to conclude that de novo hypertension in HTRs extends beyond cyclosporine-related mechanisms.

Our laboratory has focused upon the putative role of extracellular fluid volume expansion in transplant hypertension. Extracellular fluid volume expansion (~12%) is well documented in HTRs who become hypertensive (7–10). Theoretically, a fluid volume increase of only 3% to 4% can result in sustained hypertension (11). In a previous study, we acutely infused isotonic saline and determined that blood pressure in HTRs is highly salt sensitive (8). We also observed that HTRs have blunted diuretic and natriuretic responses to fluid volume expansion that may be mediated by a failure to reflexly suppress the renin-angiotensin-aldosterone system (RAAS), secondary to cardiac denervation (8).

The purpose of this study was to determine whether salt and water retention in HTRs are caused by an inability to suppress the RAAS when these patients become hypervolemic. We hypothesized that pharmacologic suppression of the RAAS would normalize renal salt and water handling in

See page 433
HTRs. We further speculated that improved salt and water elimination would lower systemic blood pressure. Using a crossover design, we measured blood pressure, renal, and endocrine responses to acute extracellular fluid volume expansion in HTRs under standardized conditions both before and after angiotensin-converting enzyme inhibition (ACEi) with captopril (Capoten). Responses in HTRs before and after ACEi were compared with responses in liver-transplant recipients (LTRs), who served as both cyclosporine and cardiac-innervated control subjects.

**METHODS**

**Subjects.** Eight male HTRs (57 ± 6 years of age) were entered into the study 22 ± 4 months (group mean) after transplantation. The HTRs were clinically stable and free from significant rejection, infection, or other major illness and all received immunosuppressive therapy with cyclosporine, prednisone, and azathioprine. The HTRs were receiving maintenance prednisone of 5 to 10 mg/day at the time of the study, and no HTRs required enhanced glucocorticoid doses within six months of the study. All HTRs had biatrial anastomosis at transplantation. The HTRs underwent endomyocardial biopsy within two months of the study, and there was no evidence of rejection at the time of biopsy in any of the subjects. Whole-blood cyclosporine trough levels, calculated as an average of four determinations over six months before the study were 225 ± 18 ng/ml (group mean). None of the transplant recipients were hypertensive before transplantation, but all required antihypertensive agents after transplantation. No HTRs had evidence of cardiac allograft vascular disease. Six male LTRs (52 ± 2 years of age) immunosuppressed with cyclosporine were also recruited to participate in the study as both cyclosporine and cardiac-innervated control group. Whole-blood cyclosporine trough levels, calculated as an average of four determinations over six months before the study, were similar in the heart (225 ± 18 ng/ml) and liver (241 ± 31 ng/ml) transplant recipients.

**Study design.** This was a crossover design. Blood pressure, renal, and endocrine responses to acute extracellular fluid volume expansion were measured in the same cohort of HTRs and LTRs under two conditions: study period 1—after discontinuation of all diuretics and antihypertensive agents; and study period 2, after a 14-day regimen of ACEi consisting of a target dose of 225 mg/24 h of captopril. The two study periods were separated by 16 ± 5 weeks (mean ± SD). The protocol was approved by the Institutional Review Board for the protection of human subjects at the University of Florida and all subjects provided written informed consent to participate in the study.

**Protocol without captopril.** Diuretics and all antihypertensive agents were discontinued 10 days before the study. Antihypertensive agents included calcium channel blockers (eight of eight HTRs), diuretics (four of eight HTRs), and ACEi (two of eight HTRs). No alpha- or beta-blockers or other cardiac medications were used by the transplant recipients. Subjects were admitted to the clinical research center (CRC). The first three days in the CRC consisted of an equilibration to a diet that consisted of sufficient calories to maintain current weight and provided 87 mEq/24 h of Na+ and 80 mEq/24 h of K+. Water intake was standardized to 1,000 ml on day 3 but provided ad libitum on all other days of the study. Consumption of alcohol, caffeine, and tobacco products was not allowed.

Subjects were awakened each morning at 7:00 AM and, while still supine, blood pressure was recorded in triplicate using an automated system (Datascope Corp., Paramus, New Jersey). Blood samples were drawn from an indwelling venous catheter for analysis of plasma concentrations of arginine vasopressin (AVP), angiotensin II (ANG II), aldosterone (ALDO), and atrial natriuretic peptide (ANP). A 24-h urine collection was started each morning at 7:00 AM. All subjects had ≥15% of Na+ balance before the saline infusion studies conducted on day 4.

On day 4, the blood pressure, renal, and endocrine responses to saline infusion were determined. At 7 AM the subjects were awakened, blood samples were obtained, and the subjects then stood and voided. Creatinine clearance was determined from the 24-h urine sample. After 1 h, the subject again voided and returned to the supine posture and remained supine until completion of the infusion. Isotonic saline (0.154 mol/l) was infused at 8 ml/kg/h for 4 h. Blood pressure, heart rate, and blood samples were drawn before the infusion and at 30, 60, 120, and 240 min during the infusion. Urine was collected at 2 and 4 h during the infusion. After completion of the infusion, an 8-h urine collection was started. Thereafter, 12-h collections were performed until the completion of the study. The 7-AM sample collection regimen described earlier was repeated on days 5 and 6. The protocol provided 48 h of observation for blood pressure, renal salt and water elimination, and hormone levels after onset of the volume expansion stimulus. After the 48-h period of observation, the diuretic and antihypertensive agents subjects received before study entry were restarted, and the subjects were discharged from the CRC.

**Protocol with captopril.** After approximately four months (group mean 16 ± 5 weeks), the same cohort of heart (n = 8) and LTRs (n = 6) again discontinued all diuretic and...
antihypertensive agents. Subjects were then progressed through a three-week home-based regimen of ACEi that consisted of a target dose of 225 mg/day of captopril. After 14 days at the target ACEi dose, subjects were admitted a second time to the CRC. The equilibration protocol and volume expansion experiments described above were repeated for each subject. After a 48-h period of observation and data collection, diuretic and antihypertensive agents were restarted, and the subjects were discharged from the CRC.

**Blood sample collection.** Blood samples for AVP, ANG II, ALDO, and ANP were drawn into vacutainers containing ethylenediaminetetraacetic acid. Plasma was separated by centrifugation at 3,500 g at 4°C until the assays were performed.

**Neurohormone analyses.** The plasma volume and neurohormone assays used in the present study have been used reliably in our laboratory for numerous experiments with human subjects (8–10,12,13). Plasma AVP was measured by radioimmunoassay as previously described (14). Plasma ANG II and ALDO levels were determined by radioimmunoassay as previously described by Braith et al. (12). Atrial natriuretic peptide was extracted from plasma using a modification of a technique described by Braith et al. (13). Radioimmunoassay was performed with a kit from Peninsula Laboratories (Belmont, California) using ANP antiserum that has 0% cross-reactivity with human brain natriuretic peptide and C-type natriuretic peptide.

**Electrolyte and creatinine analyses.** Sodium and potassium concentrations in urine and plasma were measured using a Nova 1 electrolyte analyzer (Nova Biomedical, Waltham, Massachusetts). Plasma osmolality was measured with a vapor pressure osmometer (Westcor, Logan, Utah). Standard methods were used for the measurement of serum creatinine and the calculation of renal creatinine clearance.

**Hemodynamics and left ventricular function.** To evaluate the possible contribution of cardiac function on fluid volume status, HTRs underwent right heart catheterization using standard thermodilution techniques and two-dimensional echocardiography. These examinations were all performed during the interval between the two CRC admissions.

**Statistical analysis.** Hemodynamic, renal, and hormonal responses in HTRs to the saline infusion before and after ACEi and in liver transplant control subjects before and after ACEi were compared using analysis of variance with repeated measures and the Scheffé F test. Analysis of variance was performed using the SAS general linear model procedure (SAS Institute, Cary, North Carolina). An alpha level of p < 0.05 was required for statistical significance.

### RESULTS

**Clinical status.** There were no changes in clinical status for any HTR or LTR between the two admissions to the CRC. There were no significant changes in body weight between the protocol without captopril (94.3 ± 6 kg) and the protocol with captopril (93.1 ± 7 kg). The immunosuppression regimen was not changed for any subject during the study.

**Cardiac function.** The hemodynamic and left ventricular function data in HTRs are presented in Table 1. The mean cardiac index was 2.80 ± 0.53 l/min/m², which falls within the lower one third of the normal range of 2.4 to 4.2 l/min/m² (15). Right atrial pressure for the group was slightly elevated at 6.1 ± 1.0 mm Hg, which was 22% greater than the accepted maximal right atrial mean pressure of 5 mm Hg, whereas the mean occluded pulmonary artery pressure of 10.3 ± 3.2 mm Hg fell within the stated normal range of 2 to 12 mm Hg (15). The results of our hemodynamic measures are consistent with previous reports involving HTRs (16).

**Blood pressure responses.** Baseline systolic and diastolic blood pressure values in HTRs with ACEi (131 ± 8/84 ± 2 mm Hg) were significantly lower (p ≤ 0.05) than in HTRs without ACEi (152 ± 12/96 ± 4 mm Hg). Baseline systolic and diastolic blood pressure in HTRs with ACEi was not significantly different (p ≥ 0.05) from pressure values in LTRs either with (129 ± 10/80 ± 11 mm Hg) or without ACEi (133 ± 16/82 ± 13 mm Hg). Baseline blood pressure differences in LTRs before and after ACEi were not significant (p ≥ 0.05). During the 4-h saline infusion,

### Table 1. Hemodynamics and Left Ventricular Function at Supine Rest in HTRs (n = 8)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mean RAP (mm Hg)</th>
<th>Mean PCW (mm Hg)</th>
<th>CI (l/min/m²)</th>
<th>IV Septum (mm)</th>
<th>PW (mm)</th>
<th>EF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>18</td>
<td>3.73</td>
<td>8</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>10</td>
<td>3.43</td>
<td>10</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>14</td>
<td>2.48</td>
<td>9</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>8</td>
<td>2.39</td>
<td>11</td>
<td>11</td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>4</td>
<td>3.40</td>
<td>8</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>13</td>
<td>1.90</td>
<td>10</td>
<td>10</td>
<td>58</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>8</td>
<td>1.96</td>
<td>9</td>
<td>9</td>
<td>55</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>7</td>
<td>3.10</td>
<td>9</td>
<td>10</td>
<td>60</td>
</tr>
</tbody>
</table>

Mean ± SD 6.1 ± 1.0 10.3 ± 3.2 2.80 ± 0.53 9.2 ± 1.0 10.0 ± 0.5 57.1 ± 3.7

CI = cardiac index; EF = ejection fraction; HTRs = heart transplant recipients; IV Septum = ventricular septal thickness; PCW = pulmonary capillary wedge pressure; PW = posterior ventricular wall thickness; RAP = right atrial pressure.
systolic and diastolic pressure did not change (p ≥ 0.05) in LTRs either before or after ACEi therapy (Fig. 1). In contrast, HTRs without ACEi increased (p ≤ 0.05) both systolic (+16 ± 5 mm Hg) and diastolic pressure (+9 ± 3 mm Hg) progressively throughout the infusion and remained elevated (p ≤ 0.05) at 48-h postinfusion. Systolic and diastolic pressure increased mildly during the 4-h saline challenge in HTRs receiving ACEi, but the changes were not statistically different (p ≥ 0.05) from baseline values. Blood pressure in HTRs receiving ACEi was returned to baseline values by 24 h after saline infusion.

Renal responses. Urine volume and urinary salt excretion in HTRs during the 24 h preceding the plasma volume measurements were not significantly different (p ≥ 0.05) before (0.9 ± 0.2 ml/kg/h; 3.6 ± 0.2 mEq/h) compared with after ACEi (0.8 ± 0.3 ml/kg/h; 3.4 ± 0.2 mEq/h). Likewise, serum creatinine levels and 24 h creatinine clearance in HTRs were not significantly different before (1.6 ± 0.4 mg/dl; 80.0 ± 23 ml/min) compared with after ACEi (1.5 ± 0.5 mg/dl; 82.0 ± 19 ml/min).

Urine volume during the 4-h saline infusion increased similarly (p ≥ 0.05) in HTRs and LTRs both before and after ACEi (Fig. 2). However, urine volume at 12 and 24 h after the infusion was significantly (p ≤ 0.05) diminished in HTRs without ACEi when compared with HTRs with ACEi and LTRs both with and without ACEi. Urine volume in liver patients after the saline infusion was not different (p ≥ 0.05) before and after ACEi. The net urine output over 48 h in LTRs was 82 ± 15 ml/kg with ACEi and 80 ± 17 ml/kg without ACEi. The net urine output over 48 h in HTRs was significantly greater (p ≤ 0.05) with ACEi (78 ± 11 ml/kg) than without ACEi (64 ± 14 ml/kg).

Urinary salt elimination in LTRs during the 4-h saline infusion was the same with and without ACEi (Fig. 2). In HTRs before ACEi, however, natriuresis was significantly less (p ≤ 0.05) during the 4-h saline infusion and remained significantly diminished (p ≤ 0.05) until 36 h after the saline infusion when compared with HTRs with ACEi and LTRs with or without ACEi. A delayed natriuretic re-

Figure 1. Changes (8) in blood pressure during a 4-h infusion of isotonic saline and the 48-h period after the infusion in heart- (HTR) and liver-transplant recipients (LTR) both before (W/O ACEi) and after stabilization (With ACEi) on captopril (225 mg/day). Data are mean value ± SEM. *p ≤ 0.05 versus baseline value. †p ≤ 0.05 HTRs before captopril versus HTRs after captopril and LTR both before and after captopril. ACEi = angiotensin-converting enzyme inhibition.

Figure 2. Changes in urine flow rate (UV) and urinary salt excretion (UaNa) during a 4-h infusion of isotonic saline and the 48-h period after the infusion in heart- (HTR) and liver-transplant recipients (LTR) both before (W/O ACEi) and after stabilization (With ACEi) on captopril (225 mg/day). Data are mean value ± SEM. *p ≤ 0.05 HTRs before captopril versus HTRs after captopril and LTR both before and after captopril. ACEi = angiotensin-converting enzyme inhibition.
beats/min) and after ACEi (62 ± 8 to 57 ± 3 beats/min) in liver transplant recipients (LTR) without ACEi (79 ± 7 to 80 ± 8 beats/min).

However, heart rate remained constant throughout the 48-h postinfusion observation period in HTRs both with (80 ± 7 to 80 ± 9 beats/min) and without ACEi (79 ± 7 to 80 ± 8 beats/min).

**DISCUSSION**

**Principal findings.** There were two main findings of this study: 1) Short-term pharmacologic suppression of the RAAS with high-dose captopril (225 mg/day) eliminated avid salt and fluid retention in HTRs. Before ACEi, HTRs demonstrated blunted and abnormally abbreviated diuresis.
and natriuresis in response to acute fluid volume expansion with isotonic saline. After ACEi, volume expansion elicited brisk diuretic and natriuresic responses in HTRs. Before ACEi, HTRs eliminated 50% of the salt load in 48 h. After ACEi, HTRs eliminated 86% of the salt load in 48 h. 2) HTRs are salt sensitive but defects in blood pressure homeostasis during acute hypervolemia are normalized with high-dose ACEi. Indeed, baseline systolic and diastolic pressures in HTRs receiving ACEi were not significantly different (p ≥ 0.05) from pressure values in normotensive LTRs either with or without ACEi. Additionally, systolic and diastolic pressure increased (p ≤ 0.05) during saline infusion in HTRs before ACEi but not after high-dose ACEi therapy. Our conclusions are strengthened by the crossover study design with the same cohorts of HTRs and LTRs studied before and after high-dose ACEi. Moreover, defects in blood pressure and fluid homeostasis were not observed in our control group of LTRs despite the fact that they were receiving doses of cyclosporine comparable to the HTRs. Rather, our findings indicate that the moderate fluid retention that we have documented in clinically stable HTRs who become hypertensive (7–10) is not due to cyclosporine side effects alone but may be due to a failure to reflexively suppress the RAAS during hypervolemia.

Clinical relevance. Hypertension is a frequent complication of heart transplantation attributed to cyclosporine-induced nephrotoxicity and endothelial dysfunction (1–4). However, viewing the transplanted heart as a deafferented volume-sensing organ provides an alternative explanation for the incidence and severity of hypertension in HTR. Extracellular fluid volume expansion (12% to 15%) is well documented in clinically stable HTRs who become hypertensive (7–10). Failure to reflexively suppress the RAAS...
when HTRs become hypervolemic may be responsible, in part, for the unique severity of de novo post-transplant hypertension in some HTRs (10). In the present study, infusion of 0.154 mol/l saline at 8 ml/kg/h for 4 h elicited a hypertensive response (16 ± 5/9 ± 3 mm Hg), which persisted for 48 h in HTRs without ACEi. Moreover, saline infusion did not reflexively suppress AVP and the RAAS in HTRs without ACEi. Net urine flow and urinary Na⁺ excretion was blunted and delayed. In contrast, these defects in blood pressure and fluid homeostasis were not observed in LTRs receiving comparable doses of cyclosporine and they were not observed in HTRs after pharmacologic suppression of the RAAS with high-dose ACEi therapy.

Diuretic therapy may be ineffective at mitigating fluid retention in HTRs. Initial decreases in plasma volume, glomerular filtration rate, and renal blood flow return to pretreatment values within 6 to 12 weeks of continuous hydrochlorothiazide and furosemide therapy (17,18). Initial doses of diuretic agents increase urine flow rate and renal Na⁺ excretion and decrease body weight. However, with repeated administration, progressive losses of salt and water are soon curtailed. This diuretic braking phenomenon appears to be independent of the class of diuretic agent given and is achieved by a combination of renal Na⁺ retention in the postdiuretic period and by resistance to the natriuretic response to the diuretic agent (18).

This study was not designed to evaluate the effects of long-term high-dose ACEi therapy. It is possible that HTRs receiving prolonging high-dose ACEi therapy could experience ACE escape, thereby rendering ACEi less effective at mitigating fluid retention. Moreover, ALDO expression is known to continue in some patients with complete inhibition of vascular-converting enzyme (19). ANG II generation may occur via non-ACE pathways or ANG II-independent stimulation of ALDO may occur through intravascular depletion, increased plasma potassium concentrations, corticotropin, endothelin, and catecholamines (20). Additionally, high-dose ACEi does not suppress AVP (Fig. 4). Therefore, AVP receptor antagonists may also be efficacious in the long-term clinical management of fluid retention in HTRs.

**Study limitations.** This study was limited by a small sample size and the sequential order in which HTRs participated in the two in-patient acute volume expansion studies. However, despite the small sample and nonrandomized order of experiments with and without ACEi, the study is strengthened by the crossover design wherein each subject served as their own control.

**Conclusions.** We demonstrated, in a small cohort of HTRs, that although cyclosporine may contribute to hypertension or fluid retention, the failure to reflexively suppress the RAAS in response to acute volume expansion elicits the full salt-sensitive hypertensive response. Abnormal cardio-renal reflexes, secondary to cardiac denervation, appear to be important modulators of blood pressure control and fluid volume homeostasis in HTRs. Pharmacologic suppression of the RAAS with high-dose ACEi (225 mg/day) eliminated avid salt and fluid retention and the hypertensive response to acute hypervolemia.

**Reprint requests and correspondence:** Dr. Randy W. Braith, P.O. Box 118206, Center for Exercise Science, University of Florida, Gainesville, Florida 32611. E-mail: rbraith@hhp.ufl.edu.

**REFERENCES**