Effects of long-term captopril and L-arginine treatment on ventilation and blood pressure in obese male SHHF rats

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Schlenker, E. H., C. K. Kost, Jr., and M. M. Likness. Effects of long-term captopril and L-arginine treatment on ventilation and blood pressure in obese male SHHF rats. J Appl Physiol 97: 1032–1039, 2004. We investigated the effects of captopril (Cap) and L-arginine (Arg) on hypertension and cardiopulmonary function. Our hypothesis was that Cap therapy or Arg will improve cardiopulmonary risk factors for hypertension and hypoventilation in the obese spontaneously hypertensive heart failure rat, which is characterized by hypertension, obesity, and disorders of lipid and carbohydrate metabolism. For the first study, one group of rats received Cap in drinking water, and a second group received deionized water (DI). For the second study, rats were further subdivided. Some Cap-treated rats continued on this treatment, and the other half were given DI to determine whether there would be residual effects of Cap treatment. A subgroup of rats who had received DI was then given Arg, whereas the rest remained on DI. In the first study, Cap-treated rats exhibited decreases in systolic and diastolic blood pressures, frequency of breathing, and minute ventilation, but ventilatory control was maintained. In contrast, blood pressures and relative ventilation to metabolism were higher in the DI-treated group. Removal of Cap increased blood pressure and decreased tidal volume while these rats maintained frequency. Although Arg-treated rats did not exhibit a decrease of blood pressure, ventilation was maintained in this group by preserving tidal volume. Thus Cap and Arg affected ventilation through different mechanisms independent of blood pressure.

OBSERVATIONS

spontaneously hypertensive heart failure rat; hypertension; metabolic syndrome X; ventilatory equivalent; frequency

Obesity, diabetes, and hypertension are major risk factors for cardiopulmonary and renal disease (1, 7, 9, 18, 41). The concurrence of these risk factors is also known as the metabolic syndrome X (8, 49). In addition to metabolic and cardiovascular complications, individuals with metabolic syndrome X can also present with hypoventilation during sleep and obstructive sleep apnea (8, 47).

Hypertension and congestive heart failure originate from the activation of multiple neuroendocrine systems, including the renin-angiotensin (Ang), endothelin, and atrial natriuretic peptide systems (13, 23–25, 41, 51). One in four adults develops hypertension and is at increased risk of stroke, coronary disease, congestive heart failure, and end-stage renal disease (2). Ang-converting enzyme (ACE) inhibitors are regarded as the first line of hypertensive therapy in diabetic patients due to their protective effects on the cardiovascular and renal systems (23, 27, 45). Ideally, treatment of hypertension would normalize blood pressure early in the disease, thus decreasing the incidence of cardiovascular-associated morbidity and mortality. Another agent that has been shown to decrease blood pressure is L-arginine (Arg) (50), a substrate for the production of nitric oxide (NO). In diabetic patients with hypertension, endogenous inhibitors of NO synthase (NOS) can decrease the production of NO that acts as a vasodilator (16, 39). In addition to effects on the vasculature, NO affects control of breathing directly by modulating neurotransmitter function in the carotid body and brain stem sites and/or possibly indirectly by decreasing blood pressure (10, 12, 22, 30, 32).

To determine the efficacy of ACE inhibitors and Arg on diabetes and hypertension, we utilized the obese male spontaneously hypertensive heart failure (SHHF)/McgGmi-fcrp rat that develops noninsulin-dependent diabetes mellitus, obesity, hypertension, heart failure, and renal dysfunction (15, 22, 28, 38). The “fcrp” designation reflects that the fatty (fa) gene mutation and corpulent (cp) obesity gene mutations are allelic. Obesity is an autosomal recessive trait expressed in only one of four pups, with the others expressing the lean phenotype. Obese male SHHF rats develop overt noninsulin-dependent diabetes mellitus with fasting hyperglycemia, hyperinsulinemia, hypercholesterolemia, hypertriglyceridemia, and abnormal glucose tolerance (28, 38). In addition, the obese SHHF rats exhibit pronounced proteinuria, renal glomerular lesions consistent with diabetic nephropathy, and clinical signs of heart failure (enlarged hearts, generalized edema, and dyspnea), and they die at an earlier age than their lean littermates (28). By 3 mo of age, obese SHHF have significantly enlarged hearts compared with age-matched normotensive rats, and at 6–8 mo of age pronounced left ventricular hypertrophy and some degree of ventricular failure develop. The ventricular failure leads to right ventricular hypertrophy and ultimately biventricular heart failure at 10–12 mo of age. The obese SHHF male rat was chosen because it closely resembles the human condition in which metabolic disorders often cluster with hypertension (38). We had several hypotheses for this study. First, we hypothesized that administration of captopril (Cap), an ACE inhibitor, would decrease blood pressure and preserve ventilation. Second, we postulated that removal of Cap after long-term treatment would have residual effects, including preventing an increase in blood pressure and preserving ventilation. Third, we hypothesized that administration of Arg to rats that had already developed hypertension would decrease hypertension and prevent the decrease in ventilation.

MATERIALS AND METHODS

Animals. Male obese SHHF rats (6 wk of age) were purchased from Charles River Genetic Models (Indianapolis, IN) and housed in the...
University of South Dakota Animal Facility where the light-dark cycle was lights on from 6:00 AM to 6:00 PM and the temperature was maintained at 75°F. Upon arrival at the facility, the rats were weighed and randomly assigned to receive deionized water (DI) or water containing the ACE-I Cap [10–100 mg·kg⁻¹·day⁻¹]; a dosing regimen consistent with other studies in rats (6, 14, 52). Animals received a constant supply of Purina Mills Formula 5008 rodent pellets. The timeline for both experiments is given below and depicted in Fig. 1. All protocols were approved by the University of South Dakota Animal Care and Use Committee.

**Ventilatory and metabolic protocol.** Ventilatory testing began with the placement of each rat into the plethsmographic apparatus, which was a clear Plexiglas cylinder measuring 22 cm in length and 15.5 cm in diameter. The front of the chamber contained three ports: one leading to a Statham low-pressure transducer, which in turn connected to the Bio-Pac data acquisition system; a port allowing air to enter the chamber and measure inspired oxygen and carbon dioxide; and a port used to measure chamber temperature with a Cole Palmer digital thermometer. The back of the plethsmograph contained two ports: one to measure airflow through the chamber with a Cole Palmer Rotameter and another to serve as a “leak” to stabilize measurements or when connected to vacuumed oxygen and carbon dioxide analyzers to measure fractional contents of the expired gases. The bottom of the cylinder is fitted with a removable plastic plate, which contained holes that allowed the animal to rest on a flat surface and drained any waste excreted during testing.

After placement into the apparatus, the rat was allowed ~30 min to acclimate to the chamber. After the rat became accustomed to his surroundings and frequency of breathing (f; breaths/min) stabilized, ventilation was evaluated using the barometric method used frequently in our laboratory (40). The Bio-Pac acquisition computer system was used to record the breathing pattern of each rat to determine f and tidal volume (Vₜ) and calculate minute ventilation (Vₑ), the product of the two previous variables. CO₂ production (VCO₂) was obtained by the equation flow rate × (expired fraction of CO₂ − the inspired fraction of CO₂) using the flow-through method.

![Age in Weeks](image)

**Data analysis.** Data analysis was composed of two-way ANOVA (treatment and time) with repeated measures. In the first study, changes that were present in both groups at each time point were determined to be genetic effects. Interactions between time and treatment were considered due to treatment. In the second set of studies in the four groups, a two-way ANOVA was used to determine pre- to posttreatment effects and to see whether these were different in the four groups. P values of <0.05 were accepted as significant.

Finally, the rat was removed from the plethysmograph, and rectal temperature (using a Sensorex thermometer-thermocouple system, Clifton, NJ) and body weight were measured. The ventilatory parameters evaluated included Vₑ, f, Vₑ, and VCO₂, and the ventilatory equivalent, a measure of how well ventilation and metabolism are matched, or the ratio of Vₑ to VCO₂ was calculated.

**Surgical protocol to implant telemetry devices.** Rats were randomly selected from within each treatment group at 17–18 wk of age and instrumented with radiotelemetry devices (model TA11PA-C40; Data Sciences International, St. Paul, MN) to monitor blood pressure and heart rate. The radiotelemetry devices were implanted during halothane anesthesia utilizing aseptic surgical techniques as described previously (48). In brief, a portion of the aorta distal to the renal arteries was exposed through a midline abdominal incision, and the catheter of the radiotelemetry device was inserted into the aorta through a puncture wound created with a 21-gauge needle. Medical-grade tissue and an adhesive cellulose fiber patch secured the catheter. The main body of the device, which contains the pressure sensor, radio transmitter, and battery, was then sutured into the abdominal wall after the midline incision was closed. Penicillin G procaine (5,000 units) and heparin (50 units) were given to the rats in postoperative intramuscular injections. Data acquisition was performed using the Dataquest LabPro software package (Data Sciences International, St. Paul, MN) and body weight were measured.

**Experimental timeline.** Male SHHF rats were obtained when they were 6 wk old, the age at which the obese SHHF designation could first be confirmed by Charles River Laboratories. The animals were weighed and randomly assigned into control (n = 16) or Cap-treatment (n = 16) groups. At 12–13 wk of age, when treatment had been administered for ~6 wk, Vₑ and VCO₂ were evaluated. The following week, each animal was placed in the metabolic chamber for 48 h to determine 24-h urine output and food and water intake. At 17–18 wk of age, a subgroup of control (n = 12) and Cap-treated (n = 14) rats was randomly selected to undergo surgical implantation of radiotelemetry devices (LabPro System, Data Sciences) to monitor heart rate and blood pressure. After implantation, these parameters were continuously recorded. The second period of ventilatory testing occurred when animals were 21–22 wk of age. Metabolic variables were evaluated at 23 wk of age.

To investigate the effects of removing Cap and adding Arg therapy, the 26-wk-old rats were subdivided into four groups: 1) rats that had been on Cap therapy since the beginning of the study (Cap-Cap; n = 10 for ventilatory studies and n = 7 for blood pressure studies), 2) rats that had been on Cap and then were given DI (Cap-DI; n = 12 for ventilatory studies and n = 7 for blood pressure studies), 3) rats that were on DI water and continued receiving DI (DI-DI; n = 10 for ventilatory studies and n = 7 for blood pressure studies), and 4) Cap-DI-treated rats that were now administered 2 gm/l Arg in DI (DI-Arg; n = 9 for ventilatory studies and n = 5 for blood pressure studies). This concentration of Arg is similar to that employed by others in rodent studies (33, 44). Based on this, we estimate that rats in this study were supplemented with ~250 mg/kg of Arg each day. Rats in the four groups were followed for 12 wk. A schematic of the experiments is shown in Fig. 1.
RESULTS

Radiotelemetry. Heart rate and blood pressures were continuously obtained from the surgically implanted devices. The data presented in this study were obtained on the day after the ventilation studies, at the same time of day, to prevent effects of circadian rhythms and animal handling from influencing the data. For the first study, there was no significant difference in heart rate with DI group values of 285 ± 13 beats/min compared with Cap group values of 291 ± 16 beats/min. Mean arterial pressure (MAP; in mmHg) was significantly different in the two groups at each time point. In the DI-treated group, MAP averaged 119 ± 2 mmHg, and in the CAP-treated group MAP was 90 ± 3 mmHg (P < 0.00001). Thus Cap treatment markedly decreased blood pressure in the SHHF model.

In the second study, Cap-Cap-treated rats had lower MAPs than DI-DI animals (97 ± 2 vs. 128 ± 2 mmHg; P < 0.001). Treatment of rats with Arg had no effect on blood pressure (prior DI mean value of 125 ± 3 mmHg compared with Arg value of 128 ± 4 mmHg). In contrast, removal of Cap allowed MAP to increase, but not to the extent noted with continual DI treatment. There were no significant effects of any treatments on heart rates (data not shown).

Body weight. In the first study, body weights of DI- and CAP-treated rats at two time periods were not significantly different due to treatment, but there was a time effect. DI-treated values for period 1 and period 2 were 476 ± 8.4 and 621 ± 8.5 g, respectively. Cap group mean values were 453 ± 10.1 and 565 ± 13.5 g, respectively. Two-way ANOVA indicated significant interaction between treatment and time [F(1,63) = 19.16; P = 0.000541]. There was a significant effect of time [F(1,63) = 19.98; P = 0.000001]. At period 2, body weight in the Cap-treated rats was significantly less than that of DI-treated rats (P < 0.002).

In the second study, there was an overall effect of time [F(3,71) = 6.8; P < 0.001], with rats in all four groups gaining weight, but there was no significant difference in weight after 12 wk (see Table 2). Thus, although Cap-treated animals tended to be smaller after 14 wk of treatment, longer treatment periods did not affect body weight.

Food intake, water intake, and urine output. In the both studies, food intake (g·kg⁻¹·day⁻¹), water intake (ml·kg⁻¹·day⁻¹), and urine output (ml·kg⁻¹·day⁻¹) were evaluated using the metabolic testing cage. Values attained during two 48-h periods 8 and 16 wk into the study did not demonstrate significant differences between DI and Cap groups but did indicate effects of time (Table 1). The two-way ANOVA yielded a P value of 0.7222 for treatment and a P value of 0.0003 for time. DI-treated rats’ food intake decreased from 66.8 ± 2.6 and 54.0 ± 1.1 g·kg⁻¹·day⁻¹ over time (P < 0.0004), whereas Cap values were 67.0 ± 1.7 and 55.7 ± 1.5 g·kg⁻¹·day⁻¹ (P < 0.0001). In a similar manner, water intake showed a time effect but no treatment effect. Water intake of the DI-treated animals was 150.0 ± 8.9 ml·kg⁻¹·day⁻¹ during period 1 and 113.6 ± 9.4 ml·kg⁻¹·day⁻¹ during period 2 (P = 0.0092). Comparable values for the Cap group were 156.6 ± 12.9 and 116.3 ± 9.2 ml·kg⁻¹·day⁻¹, respectively (P = 0.0076). The final variable measured was urine output. DI group data were 122.1 ± 9.4 and 87.4 ± 7.1 ml·kg⁻¹·day⁻¹ for periods 1 and 2, respectively (P = 0.0046), compared with Cap group data of 128.5 ± 12.8 and 91.5 ± 9.4 ml·kg⁻¹·day⁻¹, respectively (P = 0.0169). Two-way ANOVA indicated a P value of 0.7319 for period and 0.0001 for time. Thus food intake, water intake, and urine output exhibited a time, but not treatment, effect.

In the second study, food intake, water intake, and urine output dropped in all groups except for rats that remained on Cap (Table 2). Thus, without Cap, there was a further decrease in all three variables.

Body temperature, ventilation, and metabolism. The data for body temperature (°C) in the first study are presented in Table 1. There was a significant effect of time (P = 0.015) but not of treatment. Specifically, in period 2, DI-treated animals, but not Cap-treated rats, exhibited significantly lower body temperatures (P = 0.016). By contrast, in the second study, body temperatures dropped significantly in rats that continued on Cap treatment (37.4 ± 0.3 to 36.6 ± 0.2°C; P = 0.0245) but not in the other three groups (data not shown).

In the first study, there was an interaction between time and treatment for body weight-correction VE (BWVe; P = 0.028). DI-treated animals did not exhibit a significantly different BWVe at the two time points [27.9 ± 3.4 and 24.6 ± 1.3 ml/min × (100/body wt); Fig 2]. In contrast, Cap group values of 30.6 ± 3.7 and 21.5 ± 1.2 ml/min × (100/body wt) for BWVe were significant (P = 0.026). VT corrected for body weight and f can be seen in Table 1. Although there was a decrease of VT with time, treatment effects were not significant (P values were 0.068 related to treatment and 0.0005 for time). Cap decreased f over time. Period 1 mean data were 153.0 ± 3.0 breaths/min compared with 134 ± 4.0 breaths/min in period 2 (P = 0.0003). Thus treatment of SHHF with Cap decreased VE predominantly by decreasing f.

Table 1. Physiological characteristics of SHHF rats in study 1

<table>
<thead>
<tr>
<th></th>
<th>Period 1 DI</th>
<th>Period 2 DI</th>
<th>Period 1 Cap</th>
<th>Period 2 Cap</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT</td>
<td>38.1 ± 0.2*</td>
<td>37.5 ± 0.2*</td>
<td>37.7 ± 0.2*</td>
<td>37.3 ± 0.2*</td>
</tr>
<tr>
<td>O₂ consumption</td>
<td>24.1 ± 1.5*</td>
<td>14.0 ± 1.1*</td>
<td>23.0 ± 1.8*</td>
<td>15.3 ± 1.2*</td>
</tr>
<tr>
<td>Food intake</td>
<td>66.8 ± 2.6*</td>
<td>54.0 ± 1.1*</td>
<td>67.0 ± 1.7*</td>
<td>55.7 ± 1.5*</td>
</tr>
<tr>
<td>Water intake</td>
<td>150.0 ± 8.9*</td>
<td>113.6 ± 9.4*</td>
<td>156.6 ± 12.9*</td>
<td>116.3 ± 9.2*</td>
</tr>
<tr>
<td>Urine output</td>
<td>122.1 ± 9.4*</td>
<td>84.7 ± 7.1*</td>
<td>125.8 ± 12.8*</td>
<td>91.5 ± 9.4*</td>
</tr>
<tr>
<td>Tidal volume</td>
<td>1.78 ± 0.22*</td>
<td>1.73 ± 0.10*</td>
<td>1.98 ± 0.21*</td>
<td>1.61 ± 0.07*</td>
</tr>
<tr>
<td>Frequency</td>
<td>157.0 ± 3.0*</td>
<td>146.0 ± 0.6*</td>
<td>153.0 ± 3.0*</td>
<td>134.0 ± 4.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Body weight-corrected O₂ consumption (1,000 ml·g⁻¹·min⁻¹), body temperature (BT; °C), food intake (g/kg·day⁻¹), water intake (ml·kg⁻¹·day⁻¹), body weight-corrected tidal volume (1,000 ml/g), and frequency of breathing (breaths/min) of SHHF rats given control (deionized water; DI; n = 18) and captopril (Cap; n = 15) treatments are shown. Body weight-corrected tidal volume and frequency of breathing data in each subset were recorded when the rats were 12–13 wk of age (period 1) and then again at 21–22 wk of age (period 2). The DI group contained 18 test subjects, and the Cap group had 15 animals. O₂ consumption and body temperature data in each subset were recorded when the rats were 12–13 wk of age and then again at 21–22 wk of age. Metabolic factors were recorded the subsequent week. Lower food and water intake and urine outputs are not the results of treatment. Significant differences between each group’s treatment and time.
In the second study, there was a significant effect of treatment \( F(3,71) = 211; P < 0.0001 \) on BWVs (Fig. 3). Rats that received DI had lower values than those on Cap or Arg. This included rats that had previously received DI or Cap. In contrast, rats that remained on Cap or received Arg maintained their ventilation over time. Of interest is that rats currently receiving Cap and those that had gotten Cap for several weeks but were now on DI retained a decreased \( f \) relative to rats on DI or Arg (Table 3). Both Arg- and Cap-treated rats maintained body weight-corrected \( V_t \), unlike the drop in body weight-corrected \( V_t \) of the two groups currently receiving DI. Thus continuing Cap treatment or receiving Arg prevented the relative drop in ventilation, and rats exposed to Cap had lower \( f \) than DI- or ARG-treated rats.

In the first study, there was no significant effect of the treatment on weight-corrected \( V_{\text{CO}_2} \) although there were decreases over time (control: \( P < 0.0001; \) Cap: \( P = 0.0003 \)). To determine whether ventilation and metabolism were matched, the ventilatory equivalent (VE/\( V_{\text{CO}_2} \)) was calculated (Fig. 4). There was a significant interaction between time and treatment (\( P = 0.031 \)) and effect of time (\( P < 0.00002 \)). DI-treated rats exhibited an increase in ventilatory equivalent over time (\( P = 0.0003 \)), whereas the Cap-treated group showed no effect of time (\( P = 0.165 \)). When DI- and Cap-treated animals’ ventilatory equivalents were compared during the second time point, there was a significantly greater ventilatory equivalent noted in the DI- vs. CAP-treated group (\( P = 0.042 \)). Thus Cap treatment appeared to prevent the time-dependent increase of ventilatory equivalent in the SHHF rats.

In the second study, ventilatory equivalents exhibited a treatment effect \( F(3,71) = 34.1, P < 0.0001 \); Fig. 5). In both groups on DI, the ventilatory equivalents dropped due to a decrease in ventilation. In contrast, VE/\( V_{\text{CO}_2} \) in Cap-Cap and DI-Arg rats did not decrease, although there was greater

### Table 2. Body weights and metabolic cage data from the 2nd study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight, g</th>
<th>Fluid Intake, ml/kg·day(^{-1})</th>
<th>Food Intake, g/kg·day(^{-1})</th>
<th>Urine Output, ml/kg·day(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI-DI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>634±9.8</td>
<td>130.6±13.7</td>
<td>54.5±1.5</td>
<td>98.8±9.6</td>
</tr>
<tr>
<td>M3</td>
<td>768.8±7.9*</td>
<td>79.3±7.6*</td>
<td>45.7±1.7*</td>
<td>57.1±5.1*</td>
</tr>
<tr>
<td>DI-Arg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>636.7±8.7</td>
<td>119.4±13.7</td>
<td>55.1±1.3</td>
<td>96.7±12.3</td>
</tr>
<tr>
<td>M3</td>
<td>768.1±7.9*</td>
<td>81.4±4.6*</td>
<td>40.3±1.6*</td>
<td>62.7±4.2*</td>
</tr>
<tr>
<td>Cap-DI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>587.8±18.7</td>
<td>121.8±13.3</td>
<td>53.9±2.2</td>
<td>97.0±13.3</td>
</tr>
<tr>
<td>M3</td>
<td>729.9±24.7*</td>
<td>80.9±9.5*</td>
<td>44.7±2.5*</td>
<td>60.9±8.1*</td>
</tr>
<tr>
<td>Cap-Cap</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>578.2±19.3</td>
<td>126.6±7.5</td>
<td>52.0±45.5</td>
<td>99.7±7.3</td>
</tr>
<tr>
<td>M3</td>
<td>704.9±34.2*</td>
<td>110.1±16.8</td>
<td>45.5±1.6</td>
<td>86.7±15.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. M2, metabolic measurement period 2; M3, metabolic measurement period 3. DI-DI, rats that received DI water at both time points (\( n = 9 \)); DI-Arg, rats that received DI water but then received \( l \)-arginine in M3 (\( n = 11 \)); Cap-DI, rats that received captopril in M2 and DI in M3 (\( n = 9 \)); Cap-Cap, rats that received captopril in M2 and in M3.

*Significant effects (\( P < 0.02 \) to \( P = 0.0001 \)) of M3 relative to M2 treatments. Note that only rats receiving captopril at both time points exhibited no change in fluid intake, food intake, or urine output from M2 to M3.

Fig. 3. Body weight-corrected \( V_t \) (\( V_t \times (100/body weight) \)) of SHHF rats in the second study. Rats received either DI or Cap during the first period and then were divided into 4 groups: DI and then DI (DI-DI), DI and then \( l \)-arginine (DI-Arg), Cap and then DI (Cap-DI), or Cap and then continued Cap (Cap-Cap). Values are means ± SE. *Significant decreases (\( P < 0.01 \)) in \( V_t \) between treatments at periods 1 and 2.

### Table 3. Body weight-corrected tidal volume and frequency in study 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tidal Volume, 1,000 ml/g</th>
<th>Frequency, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI-DI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>1.69±0.11</td>
<td>150±12</td>
</tr>
<tr>
<td>R3</td>
<td>1.11±0.06*</td>
<td>142±11</td>
</tr>
<tr>
<td>DI-Arg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>1.52±0.13</td>
<td>146±4</td>
</tr>
<tr>
<td>R3</td>
<td>1.33±0.15</td>
<td>144±8</td>
</tr>
<tr>
<td>Cap-DI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>1.69±0.08</td>
<td>135±4†</td>
</tr>
<tr>
<td>R3</td>
<td>1.35±0.12*</td>
<td>131±8†</td>
</tr>
<tr>
<td>Cap-Cap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>1.60±0.05</td>
<td>137±5†</td>
</tr>
<tr>
<td>R3</td>
<td>1.33±0.12</td>
<td>132±10†</td>
</tr>
</tbody>
</table>

Values are means ± SE. R2, respiratory measurement period 2; R3, respiratory measurement period 3. *Significant effects of treatments from R2 to R3. Breathing frequencies of rats receiving or who had received captopril were significantly less (†\( P < 0.01 \)) than rats with a prior history of DI or that received arginine.

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*Fig. 2. First study body weight-corrected minute ventilation (\( V_t \)) values, obtained by the equation ml/min × (100/body wt), of SHHF rats given DI (\( n = 18 \)) and Cap (\( n = 15 \)) treatments were recorded in each subset initially when the rats were 12–13 wk of age and then again at 21–22 wk of age. Values are means ± SE. *Significant difference between Cap R1 and Cap R2 values.

*Fig. 3. Body weight-corrected \( V_t \) (\( V_t \times (100/body weight) \)) of SHHF rats in the second study. Rats received either DI or Cap during the first period and then were divided into 4 groups: DI and then DI (DI-DI), DI and then \( l \)-arginine (DI-Arg), Cap and then DI (Cap-DI), or Cap and then continued Cap (Cap-Cap). Values are means ± SE. *Significant decreases (\( P < 0.01 \)) in \( V_t \) between treatments at periods 1 and 2.

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*Fig. 4. First study body weight-corrected minute ventilation (\( V_t \)) values, obtained by the equation ml/min × (100/body wt), of SHHF rats given DI (\( n = 18 \)) and Cap (\( n = 15 \)) treatments were recorded in each subset initially when the rats were 12–13 wk of age and then again at 21–22 wk of age. Values are means ± SE. *Significant difference between Cap R1 and Cap R2 values.
variability of responses in the group continuing on Cap relative to those receiving Arg treatment \( F(1,35) = 6.87; P = 0.021 \).

**Periodic breathing.** Abnormal breathing patterns were observed in both studies (Fig. 6). A particular pattern could continue for the entire recording. These instances of abnormal breathing patterns most often consisted of several seconds absent of ventilation interrupted by a single sharp breath. Other abnormal breathing patterns included an apnea for 15–20 s interspersed by a sharp cluster of rapid breaths. Analysis of frequency, treatment group, and age at which the rats exhibited irregular breathing revealed a 12.5% occurrence. These patterns were noted after the second recordings in study 1 and also during study 2. Period 1 revealed no such events, suggesting that irregular breathing may be an age-related factor in the obese SHHF rat.

**DISCUSSION**

The first study assessed the effects that long-term administration of Cap had on ventilatory, metabolic, and cardiovascular parameters in male obese SHHF rats. Cap treatment produced significant decreases in blood pressure, \( f \), \( V\dot{E} \), and maintenance of the ventilatory equivalent. No effects of Cap were noted on \( V\dot{CO}_2 \), although this variable decreased with time in DI-treated rats.

In the second study, removal of Cap allowed MAP to rise, but not to the levels noted in DI-treated rats. Treatment of rats with Arg did not affect MAP. Ventilation was conserved with either Cap treatment or Arg by maintaining \( Vr \). Rats that received Cap or had this treatment removed exhibited lower \( f \) without showing significantly lower body weights.

**Blood pressure.** Blockade of the renin-Ang system is an effective means of controlling hypertension and managing congestive heart failure (5, 19, 23). As expected with the SHHF model, the DI-treated group increased MAP over time. Moreover, this effect was prevented in CAP-treated male obese SHHF rats. These results are similar to findings in previous studies in SHHF rats (5). The exact mechanism of the increase in hypertension in this model is not known and could be caused by any individual factor or combination of factors, including increased body weight, leptin resistance, increased salt sensitivity, increased levels of Ang II, or reduced levels of bradykinin and NO (3, 16, 24, 27, 36, 37, 39).

Mechanisms by which Cap may decrease blood pressure include decreased production of Ang II and increased levels of bradykinin and NO (45). NO is produced through the transformation of Arg to \( L\)-citrulline by the NOS enzyme family. NO is an important factor regulating vascular tone (50), renal sodium excretion, and the pressure-diuresis-natriuresis response (9, 45) and therefore arterial blood pressure (39). Increased NO production decreases hypertension. The fact that Arg-treated SHHF rats in the present study did not exhibit a decrease in blood pressure may suggest that the effects of Cap on blood pressure may be through other mechanisms, that there is a strain difference in response to NO systems as noted in several previous studies (12, 34, 43) or that treatment was
started when elevated blood pressure became established. To test the last possibility, starting Arg treatment in young obese SHHF rats needs to be done to determine whether Arg can prevent the development of elevated blood pressure.

Of interest is that removal of Cap caused MAP to increase, but it never reached levels noted in DI-treated rats. A similar finding was reported by Harrap et al. (21) in young spontaneously hypertensive rats (SHR) treated with the ACE inhibitor perindoril. At 25 wk of age, SHR that had been treated with the ACE inhibitor for 4 wk during an early age also showed a reduced total peripheral resistance due to decreased media to lumen ratios in mesenteric resistance vessels and decreased cardiac hypertrophy. Thus the attenuation of the return of blood pressure to levels noted in untreated SHR or obese SHHF rats may suggest that there was a remodeling of peripheral factors such as smooth muscle mass in blood vessels (21, 50) and possibly a resetting of central regulatory areas associated with regulation of blood pressure noted in other studies (20, 26).

**Ventilation.** Treatment of rats with Cap (in both studies) and Arg (in the second study) maintained ventilation by preventing a significant decrease in Vt. Of interest is that both long-term Cap treatment and removal of Cap reduced f, which was not observed in Arg-treated rats. Thus NO production in peripheral chemoreceptors or the central nervous system alone did not solely contribute to the results reported in this study; rather, a remodeling of central regulation of frequency may have occurred. Cap treatment, however, did not prevent the development of periodic breathing, a finding also described in SHR (4). Additional mechanisms by which Cap may maintain ventilation is altering reflex responses to elevated blood pressure (10). Thus the decrease of blood pressure in Cap-treated SHHF rats may be one way that ventilation was maintained relative to the increase in ventilation in DI-treated rats. Finally, hemodynamic studies in normotensive and hypertensive (SHR) rats have shown an increased cerebral blood flow in SHR (17). Moreover, antihypertensive treatment in SHR decreased cerebral blood flow (20). An increased blood flow may result in lower levels of CO₂, which may decrease ventilation. To determine whether central nervous system blood flow is altered in untreated SHHF rats and whether this change in blood flow may be related to ventilatory responses noted in the present study, additional investigations similar to those reported by Granstam et al. (17) need to be conducted.

NO exerts a role in respiratory control by enhancing the excitability of the neurons involved in the generation of central respiratory activity (22, 30, 43). Obese Zucker rats display excitability of the neurons involved in the generation of central respiratory activity (22, 30, 43). Obese Zucker rats display excitability of the neurons involved in the generation of central respiratory activity (22, 30, 43). Obese Zucker animals (30). Leptin suppresses NOS activity in the brain (3) but has a direct stimulating effect on respiratory control centers (22, 42). This suggests that the manifestation of ventilatory abnormalities in animal models of obesity derives from multifaceted interactions, including elevated blood pressure, low levels of NO, and disrupted leptin signaling.

Altered breathing patterns, such as an increase in f and decreased Vt, are noted and exhibited in several animal models of obesity. In the obese compared with lean Zucker rat, f was increased (11). Longitudinal studies indicated that mutant mice homozygote (ob/ob) for the obesity gene also developed rapid baseline breathing relative to wild-type mice (31, 35, 46). Further evidence for obesity, and not predominantly hypertension, contributing to the higher f and lower Vt pattern in DI-treated obese SHHF rats in the present study comes from a recent study of lean SHHF rats (40). Male lean SHHF in that study exhibited hypertension and had a f of 111 ± 3 breaths/min relative to 115 ± 2 breaths/min in Wistar-Kyoto normotensive control rats. Of interest is that Vt was 15% lower (P < 0.05) in lean SHHF rats than in Wistar-Kyoto rats.

Another mechanism whereby Cap may affect breathing is by either preventing or attenuating pulmonary edema. Ang I has been known to induce pulmonary edema (19, 53). An elevated f can be induced by water in the lungs. Cap may decrease f by decreasing water content in the lungs. A study elucidating the mechanism of hemodynamic pulmonary edema determined that the development of Ang I-induced pulmonary edema was significantly depressed by Cap (53). The mechanism behind this effect is that Ang I is converted to Ang II, whose effects are prevented by Cap. A study of the effect of Cap on the recovery of patients with acute pulmonary edema reported similar results. Over the first 40 min of treatment, the mean acute pulmonary edema distress scores were significantly lower for the patients given Cap (19). Reduction of fluid in the pulmonary alveoli creates less ventilatory resistance and stimulates an increase in Vt and a decrease of f, Vt, and ventilatory equivalent (19). Whether this is a mechanism responsible for the decreased f in the Cap-treated obese SHHF rats in the present study needs to be evaluated.

Another point that needs to be addressed is that, because rats were followed over a period of several months, effects of the development of the metabolic syndrome X, as well as age-related changes, may affect metabolic and cardiopulmonary variables. Thus, when the three time periods are compared in the untreated obese SHHF rats, body temperature was lower, oxygen consumption decreased, and Vt dropped, whereas blood pressure increased. In studies within our laboratory (Schlenker, unpublished observations), decreases in oxygen consumption were noted from 3 to 8 mo in male Sprague-Dawley rats; however, Vt increased, and ventilation and oxygen consumption were matched. The findings in the present study of obese SHHF male rats suggest that both age and metabolic syndrome X characteristics affected these variables.
In summary, treatment of male obese SHHF rats with Cap produces a decrease of blood pressure, f, Vt, and ventilatory equivalent. Removal of Cap maintained f and allowed blood pressure to increase, but not to the same extent as in DI-treated obese SHHF rats. Although Arg treatment did not show the expected decrease in blood pressure, ventilation was maintained by preserving Vt. These results suggest that Cap remodels both cardiovascular and respiratory systems and that Arg conserves ventilation independent of increased blood pressure. Future studies need to be designed to determine whether earlier administration of Arg can prevent an increase in blood pressure and maintain ventilation in obese SHHF rats. How Cap acts to decrease f needs to be investigated. Clearly, the obese SHHF is a good animal model to investigate the contributions metabolic syndrome X has to increased cardiopulmonary morbidity and mortality in human beings.

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GRANTS

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REFERENCES


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