Effects of Long-term Captopril and L-Arginine Treatment on Ventilation and Blood Pressure in Obese Male SHHF Rats

E. H. Schlenker *, C. K. Kost, Jr. and M. M. Likness

Division of Basic Biomedical Sciences, University of South Dakota School of Medicine, Vermillion, SD 57069, USA

* Corresponding Author
Phone Number: 605-677-5160
E-mail Address: eschlenk@usd.edu

Running Title: Captopril and L-Arginine Affect Breathing
Abstract

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 that 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 now given DI to determine if there would be residual effects of CAP treatment. A subgroup of rats who had received DI was then given ARG while the rest remained on DI. In the first study CAP-treated rats exhibited decreases in systolic and diastolic blood pressures, frequency of breathing, minute ventilation, and maintained ventilatory control. 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.

Key words: Hypertension, Metabolic Syndrome X, Ventilatory Equivalent, Frequency
Introduction

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 (RAS), endothelin, and atrial natriuretic peptide systems (14, 23, 25, 26, 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). Angiotensin-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, 28, 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 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, 31, 33).

To determine the efficacy of ACE inhibitors and ARG on diabetes and hypertension we utilized the obese male SHHF/MccGmi-fu10 rat that develops non-
insulin dependent diabetes mellitus (NIDDM), obesity, hypertension, heart failure, and renal dysfunction (15,22, 29,37). The “SHHF” designation reflects that the rats were selectively bred for Spontaneous Hypertension and Heart Failure, and the “fa cp” designation reflects that the fa (fatty) gene mutation and cp (corpulent) obesity gene mutations are allelic. Obesity is an autosomal recessive trait expressed in only one out of four pups, with the others expressing the lean phenotype. Obese male SHHF rats develop overt NIDDM with fasting hyperglycemia, hyperinsulinemia, hypercholesterolemia, hypertriglyceridemia, and abnormal glucose tolerance (29,37). In addition, the obese SHHF rats exhibit pronounced proteinuria, renal glomerular lesions consistent with diabetic nephropathy, clinical signs of heart failure (enlarged hearts, generalized edema, and dyspnea) and die at an earlier age than do their lean littermates (29). By three months of age, obese SHHF have significantly enlarged hearts compared to age-matched normotensive rats, and at 6-8 months of age, pronounced left ventricular hypertrophy and some degree of ventricular failure develops. The ventricular failure leads to right ventricular hypertrophy and ultimately biventricular heart failure at 10-12 months of age. The obese SHHF male rat was chosen because it closely resembles the human condition in which metabolic disorders often cluster with hypertension (37). We had several hypotheses for this study. First we hypothesized that administration of captopril, an angiotensin converting enzyme inhibitor, would decrease blood pressure and preserve ventilation. Second, we postulated that removal of captopril after long term treatment would have residual effects including preventing an increase in blood pressure and preserving ventilation. Third, we hypothesized that administration of L-arginine to rats
who had already developed hypertension would decrease hypertension and prevent the
decrease in ventilation.

**Materials and methods**

**Animals** Male obese SHHF rats (6 weeks 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 de-ionized water or water containing the
ACE-I, captopril (approximately 100 mg kg⁻¹ day⁻¹); a dosing regimen consistent with
other studies in rats (6,13, 52). Animals received a constant supply of Purina Mills
Formulab 5008 rodent pellets. The timeline for both experiments is given below and
depicted in Figure 1. All protocols were approved by the University of South Dakota
Animal Care and Use Committee.

**Ventilatory & metabolic Protocol:** Ventilatory testing began with the placement
of each rat into the plethsmographic apparatus, 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 using a
Cole Palmer digital thermometer. The back of the plethysmograph contained two ports:
one to measure airflow through the chamber using a Gilmont 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 containing holes that allowed the animal to rest on a flat surface and drained any waste excreted during testing.

Following placement into the apparatus, the rat was allowed about 30 minutes to acclimate to the chamber. After the rat became accustomed to his surroundings and frequency of breathing 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 frequency of breathing (f, breaths per minute), and tidal volume (Vt) and calculate minute ventilation (VE), the product of 2 previous variables. CO₂ production obtained by the equation flow rate multiplied by \((F_{ECO₂}-F_{ICO₂})\) using the flow through method. Finally the rat was removed from the plethysmograph, and rectal temperature (using a Sensortek, Clifton, NJ, thermometer-thermocouple system) and body weight were measured. The ventilatory parameters evaluated included Vt, f, VE 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 weeks of age, and instrumented with radio telemetry devices (Model TA11PA-C40; Data Sciences International, St. Paul MN) to monitor blood pressure and heart rate. The radiotelemetry devices were implanted during isoflurane 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 G needle. Medical grade tissue and a cellulose fiber patch secured the catheter. The main body of the device, which contains
the pressure sensor, radio transmitter, and the battery, was then sutured into the abdominal wall after the midline incision was closed. Penicillin G procaine (5000 units) and heparin (50 units) were given to the rats in post-operative IM injections. Data acquisition was performed using the Dataquest LabPRO software package (Data Sciences International) with sampling parameters adjusted to 10-sec scan periods at 10-min intervals.

**Measurement of food and water intake recording:** Metabolic evaluations were performed at various times (indicated in Figure 1) by placing the rats in metabolic recording cages (Nalgene) for 48 hours. Food and water intake, and urine output were measured.

**Experimental Timeline:** Male SHHF rats were obtained when they were six weeks old, the age at which the SHHF designation could first be confirmed by Charles River Laboratories. The animals were weighed and randomly assigned into control (n =16) or captopril treatment (n=16) groups. At 12-13 weeks of age, when treatment had been administered for approximately eight weeks, ventilation and VCO₂ were evaluated. The following week each animal was placed into the metabolic chamber for 48 hours to determine 24 hour urine output, and food and water intake. At 17-18 weeks of age, a subgroup of control (n=12) and captopril-treated (n=13) rats were 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 weeks of age. Metabolic variables were evaluated at 23 weeks of age.
To investigate the effects of removing captopril and adding L-arginine therapy, the 26 week old rats were subdivided into 4 groups: those who had been on captopril therapy since the beginning of the study (CAP-CAP; n=10 for ventilatory studies and n=7 for BP studies), those who had been on captopril and then were given DI water (CAP-DI; n=12 for ventilatory studies and n=7 for BP studies), rats who were on DI water and continued receiving DI (DI-DI, n=10 for ventilatory studies and n=7 for BP studies) or DI-treated rats that were now administered L-arginine 2gm/L in deionized water (DI-ARG; n=9 for ventilatory studies and n=5 for BP studies). This concentration of L-arginine is similar to that employed by others in rodent studies (34, 44). Based on this, we estimate that rats in this study were supplemented with approximately 250 mg/kg of L-arginine each day. Rats in the 4 groups were followed for 12 weeks. A schematic of the experiments is shown in Figure 1.

Data Analysis: Data analysis was composed of 2 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 4 groups, a 2 way ANOVA was used to determine pre to post treatment effects and to see if these were different in the 4 groups. P-values of less than 0.05 were accepted as significant. Paired or unpaired t-tests were performed if ANOVA results were significant. Data are presented as means ± SEM.

Results

Radiotelemetry: Heart rate and blood pressures were continuously obtained from the surgically implanted devices. The data presented in this study were obtained at the
same time of day as the ventilatory studies were conducted, but on the following 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 bpm compared to CAP group values of 291±16 bpm. Mean arterial pressure (MAP in mm Hg) was significantly different in the 2 groups at each time point. In the DI-treated group MAP averaged 119 ± 2 mm Hg and in the CAP-treated group MAP was 90 ± 3 (P<0.00001) Thus, captopril treatment markedly decreased blood pressure in the SHHF model.

In the second study, CAP-CAP treated rats had lower mean arterial pressures (in mm Hg) than DI-DI animals (97± 2 versus 128 ± 2, P<0.001). Treatment of rats with ARG had no effect on blood pressure (prior DI mean value 125 ± 3 mm Hg compared to arginine 128 ± 4 mm Hg). In contrast, removal of CAP (CAP-DI) caused the mean arterial pressure to rise from 95 ± 3 to 118 ± 3, P=0.007, but not to the extent of animals treated with DI (P<0.01). Thus, captopril maintained a decrease in MAP, arginine had no effect on MAP, and removal of captopril 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 (gm) and 621±8.5 (gm), respectively. CAP-group mean values were 453±10.1 and 565±13.5 respectively. The two-way ANOVA indicated significant interaction between treatment and time (F₁, 63=19.16, P=0.000541). There was a significant effect of time (F₁, 63=19.98,
P=0.000001). At the 2nd Period, body weight in the CAP-treated rats was significantly less than that of the 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 4 groups gaining weight, but there was no significant difference in weight after 12 weeks (Table 2). Thus, although CAP-treated animals tended to be smaller after 14 weeks of treatment, longer treatment periods did not affect body weight.

**Food intake, water intake and urine output:** In the both studies food intake (g/24/Kg), water intake ml/24/Kg), and urine output (ml/24/Kg) were evaluated using the metabolic testing cage. Values that were attained during two 48-hour periods 8 and 16 weeks into the study did not demonstrate significant differences between DI and CAP groups, but did indicate effects of time (Table 1). The 2 way ANOVA yielded a P=0.7222 for treatment and a P=0.0003 for time. DI-treated rats’ food intake decreased from 66.8±2.6 and 54.0±1.1 over time (P<0.0004), whereas CAP values were 67.0±1.7 and 55.7±1.5, P<0.0001. In a similar manner, water intake showed a time effect, but no treatment effect. Water intake (ml/24 hours/Kg) of the DI-treated animals was 150.0±8.9 during the first time period and 113.6±9.4 during the second time period, P=0.0092. Comparable values for the CAP group were 156.6±12.9 and 116.3±9.2, P=0.0076. The final variable measured was urine output. DI group’s data 122.1±9.4 and 87.4±7.1, P=0.0046; compared to CAP group’s data of 128.5±12.8 and 91.5±9.4, P=0.0169. The two way ANOVA indicated P-values of 0.7319 for treatment 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 rats that remained on captopril (Table 2). Thus, without captopril there was a further decrease in all 3 variables.

**Body temperature, Ventilation, and Metabolism:** The data for body temperature (°C) in the first study is 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 captopril-treated rats, exhibited significantly lower body temperatures (P=0.016). By contrast in the second study body temperatures dropped significantly in the rats who continued on captopril treatment (37.4 ± 0.3 °C to 36.6 ± 0.2 °C, P=0.0245), but not in the other 3 groups (data not shown).

In the first study there was an interaction between time and treatment for body weight corrected minute ventilation (BWVe, P=0.028). DI-treated animals did not exhibit a significantly BWVe different at the 2 time points (27.9±3.4 and 24.6±1.3 ml/min x (100/Body Weight), Fig.2). In contrast, CAP group values of 30.6±3.7 and 21.5±1.2 ml/min x (100/Body Weight) for body weight corrected minute ventilation were significant (P=0.026). Tidal volume corrected for body weight and frequency of breathing can be seen in Table 1. Although there was a decrease of tidal volume with time, treatment effects were not significant (P-values were 0.068 related to treatment and 0.0005 for time). Captopril decreased frequency of breathing over time. Period 1 (R1) mean data (in breaths per minute) was 153.0±3.0 compared to 134±4.0 in period 2 (R2), p-value 0.0003. Thus, treatment of SHHF with captopril decreased minute ventilation due predominantly by decreasing frequency of breathing.
In the second study there was a significant effect of treatment (F3, 71=211, P<0.0001) on BWVe (Figure 3). Rats who received DI had lower values than those on captopril or L-arginine. This included rats who had previously received DI or captopril. In contrast, rats that remained on captopril or received L-arginine maintained their ventilation over time. Of interest is that rats currently receiving captopril and those who had gotten captopril for several weeks, but were now on DI, retained a decreased frequency of breathing relative to rats on DI or ARG (Table 3). Both L-arginine-treated and captopril-treated rats maintained BWVt, unlike the drop in BWVt of the 2 groups currently receiving DI. Thus, continuing captopril treatment or receiving L-arginine prevented the relative drop in ventilation, and rats exposed to captopril had lower breathing frequencies than did DI or ARG-treated rats.

In the first study there was no significant effect of the treatment on weight corrected CO2 production, although there were decreases over time (Control (P<0.0001) and Captopril (P=0.0003)). To determine if ventilation and metabolism were matched the ventilatory equivalent (VE/VCO₂) was calculated (Figure. 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- versus the CAP-treated group (P=0.042). Thus, captopril 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 (Figure 5, F 3, 71=34.1, P<0.0001). In both groups on DI, the ventilatory equivalents dropped due to a decrease in ventilation. In contrast, VE/VCO₂ in captopril (CAP-CAP) and arginine-treated (DI-ARG) rats did not decrease, although there was greater variability of responses in the group continuing on captopril relative to those receiving l-arginine treatment (F 1, 35= 6.87, P=0.021).

**Periodic Breathing.** Abnormal breathing patterns were observed in both studies (Figure 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 seconds 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 maybe an age-related factor in the obese SHHF rat.

**Discussion**

The first study assessed the effects long term administration of captopril had on ventilatory, metabolic, and cardiovascular parameters in male obese SHHF rats. Captopril treatment produced significant decreases in blood pressure, frequency of breathing, minute ventilation, and maintenance of the ventilatory equivalent. No effects of captopril were noted on carbon dioxide production, although this variable decreased with time in DI and CAP-treated rats.
In the second study, removal of captopril allowed mean arterial pressure to rise, but not to the levels noted in DI-treated rats. Treatment of rats with arginine did not affect mean arterial pressure. Ventilation was conserved with either captopril treatment or arginine by maintaining tidal volume. Rats that received captopril or had this treatment removed, exhibited lower frequency of breathing without showing significantly lower body weights.

**Blood Pressure**

Blockade of the renin-angiotensin 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 mean arterial pressure over time. Moreover, this effect was prevented in CAP-treated male obese SHHF rats. These results are similar to findings of 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 or combination of factors including increased body weight, leptin resistance, increased salt sensitivity, increased levels of angiotensin II, or reduced levels of bradykinin and nitric oxide (3, 16, 25, 28, 36, 38, 39).

Mechanisms by which captopril may decrease blood pressure include decreased production of angiotensin II, on increased levels of bradykinin and nitric oxide (45). Nitric oxide (NO) is produced through the transformation of L-arginine to L-citrulline by the NO synthases (NOS) enzyme family. NO is an important factor regulating vascular tone (50), renal sodium excretion and the pressure-diuresis -natiuresis (PDN) response (9, 45), and therefore arterial blood pressure (39). Increased nitric oxide production decreases hypertension. The fact that arginine-treated SHHF rats in the present study did
not exhibit a decrease in blood pressure may suggest that captopril’s effects 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, 24,43), or that treatment was started when elevated blood pressure became established. To test the last possibility starting L-arginine treatment in young obese SHHF rats needs to be done to determine if L-arginine can prevent the development of elevated blood pressure.

Of interest is that removal of captopril caused mean arterial pressure to increase, but it never reached levels noted in DI-treated rats. A similar finding was reported by Harrap and coworkers (21) in young spontaneously hypertensive rats (SHR) treated with the ACE inhibitor perindoril. At 25 weeks of age SHR rats that had been treated with the ACE inhibitor for 4 weeks 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 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 (20,27).

**Ventilation**

Treatment of rats with captopril (in both studies) and arginine (in the second study) maintained ventilation by preventing a significant decrease in tidal volume. Of interest is that both long term captopril treatment and removal of captopril reduced breathing frequency, results not observed in arginine-treated rats. Thus, nitric oxide production in peripheral chemoreceptors or the CNS alone did not solely contribute to the
results reported in this study; rather a remodeling of central regulation of frequency may have occurred. Captopril treatment, however, did not prevent the development of periodic breathing, a finding also described in spontaneously hypertensive rats (4). Additional mechanisms by which captopril 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 rats (17). Moreover, antihypertensive treatment in SHR rats decreased cerebral blood flow (20). An increased blood flow may result in lower levels of CO₂, which may decrease ventilation. To determine if CNS blood flow is altered in untreated SHHF rats and this change in blood flow may be related to ventilatory responses noted in the present study, additional investigations similar to those reported by Granstam and colleagues (17) need to be conducted.

Nitric oxide exerts a role in respiratory control by enhancing the excitability of the neurons involved in the generation of central respiratory activity (22, 31, 43). Obese Zucker rats display decreased NO synthase activity in the hypothalamus (30), which may contribute to obesity (3). A study of the effects of N(G)-nitro-L-arginine methyl ester (L-NAME), a nonspecific NOS inhibitor, in obese and lean Zucker rats found that during room air, obese rats breathed with a significantly higher frequency and a lower tidal volume than lean rats (31). This indicates that decreased levels of NO in the CNS may, in part, be responsible for the higher frequency and lower tidal volume breathing pattern noted in the DI-treated rats.
The obese male SHHF is homozygote (cp/cp) for a null mutation of the leptin receptor (cp). When obese male SHHF were compared with heterozygous (+/cp) and homozygous (+/+), lean male SHHF rats in response to a high salt diet, obesity secondary to leptin resistance (cp/cp) resulted in increased salt sensitivity (38). Animals receiving the high salt diet also had significantly greater systolic blood pressure. When Bosentan, an endothelin A and B receptor antagonist, was administered, it prevented the salt-induced increase of induced pressure and renal excretion of nitric oxide was doubled (38). Obese Zucker (fa/fa) rats have a defect in leptin access to the brain due to a mutation of the leptin receptor, which may be a cause of their obesity. Disrupted leptin transport into the brain may contribute to the NO dysfunction in respiratory control in obese Zucker animals (31). 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 in blood pressure and low levels of nitric oxide and disrupted leptin signaling.

Altered breathing patterns such as an increase in breathing frequency and decreased tidal volume are noted and exhibited in several animal models of obesity. In the obese compared to lean Zucker rat, frequency of breathing was increased (11). Longitudinal studies indicated that mutant mice homozygote (ob/ob) for the obesity gene also developed rapid baseline breathing relative to the wild type mice (32, 35, 46). Further evidence for obesity, and not predominantly hypertension contributing to the higher frequency, lower tidal volume 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 breathing frequency of 111 ± 3 breaths/minute relative to the 115 ± 2 breaths per minute in Wistar Kyoto (WKY) normotensive controls. Of interest is that tidal volumes were 15% lower (P<0.05) in the lean SHHF rats than WKY rats.

Another mechanism whereby captopril may affect breathing is by either preventing or attenuating pulmonary edema. Angiotensin I (ang-I) has been known to induce pulmonary edema (19, 53). An elevated breathing frequency can be induced by water in the lungs. Captopril may decrease breathing frequency 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 captopril (53). The mechanism behind this effect is that ang-I is converted to angiotensin II, whose effects are prevented by captopril. A study of captopril’s effect on the recovery of patients with acute pulmonary edema (APE) reported similar results. Over the first 40 minutes of treatment, the mean APE distress (APEX) scores were significantly lower for the patients given captopril (19). Reduction of fluid in the pulmonary alveoli creates less ventilatory resistance and stimulates an increase in tidal volume, a decrease of breathing frequency, a decrease in minute ventilation and ventilatory equivalent (19). Whether this is a mechanism responsible for the decreased frequency of breathing in the captopril-treated obese SHHF rats in the current study needs to be evaluated.

Another point that needs to be addressed is that since 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 3 time periods are compared in the untreated obese SHHF rats, body
temperature is lower and oxygen consumption decreased and tidal volume dropped, while
blood pressure increased. In studies within our laboratory (Schlenker, unpublished
observations), decreases in oxygen consumption were noted from 3 to 8 months in male
Sprague Dawley rats, however, tidal volume 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 captopril produces a
decrease of blood pressure, frequency of breathing, minute ventilation, and ventilatory
equivalent. Removal of captopril maintained frequency of breathing and allowed blood
pressure to increase, but not to the same extent than that in DI-treated obese SHHF rats.
Although arginine treatment did not show the expected decrease in blood pressure,
ventilation was maintained by preserving tidal volume. These results suggest that

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Acknowledgements

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References


Figure Legends

Figure 1. Obese SHHF male rats were obtained at 6 weeks of age, and randomly assigned to receive either deionized drinking water (DI) or captopril (CAP) in drinking water. Respiratory measures (R) were obtained at 12-13 (R1), 21-22 (R2), and 38-39 weeks of age (R3). Rats were placed in metabolism cages (M) at 14-15 (M1), 23-24 (M2) and 34-35 (M3) weeks of age. Telemetry devices (T) for blood pressure monitoring were implanted at 17-18 weeks of age. When rats were 26 weeks of age, DI-treated rats were further subdivided into two groups; continuation on DI water (DI-DI) or replacement of DI water with a solution containing L-arginine (DI-ARG). In addition, half of the captopril-treated rats were withdrawn from treatment and given DI water (CAP-DI) while the remaining rats were maintained on captopril treatment (CAP-CAP).

Figure 2. First study bodyweight-corrected minute ventilation, obtained by the equation [ml/min X (100/Body Weight)], of SHHF rats given deiodinized water (DI, n=18) and captopril (CAP, n=15) treatments was recorded in each subset initially when the rats were 12-13 weeks of age and then again at 21-22 weeks of age. The asterisk denotes a significant difference between Cap 1 and CAP2 values. Values are means ± SEM

Figure 3. Body weight-corrected minute ventilation (minute ventilation X (100/Body weight) of SHHF rats in the second study. Rats received either deiodinized (DI) water of captopril (CAP) during the first period and then were divided into 4 groups: DI and then DI, DI and then arginine (DI-ARG), Cap and then DI or CAP and then continued on
CAP. The asterisks denote significant decreases (P<0.01) in ventilation comparing first period to second period treatments. Values are means ± SEM.

**Figure 4.** First study ventilatory equivalents (VE/VCO₂) of SHHF control (DI, n=18) and captopril-treated (CAP, n=15) rats were recorded in each subset initially when the rats were 12-13 weeks of age (R1) and then again at 21-22 weeks of age (R2). The asterisk denotes significant differences in DI R1 and DI R2. Values are means ± SEM.

**Figure 5.** Second study ventilatory equivalents (VE/VCO₂) of SHHF rats that received either deiodinized (DI) water of captopril (CAP) during the first period and then were divided into 4 groups: DI and then DI (DI-DI), DI and then arginine (DI-ARG), CAP and then DI (CAP-DI) or CAP and continued on CAP (CAP-CAP). The asterisks denote significant decreases (* P<0.05, **P<0.01) in ventilation comparing first period to second period treatments. Values are means ± SEM.

Figure 6. Schematic representation of abnormal breathing patterns (A, B, and C) and normal breathing pattern in obese SHHF rats.
Table 1. Physiological Characteristics of SHHF rats in Study 1

<table>
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<tr>
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<th>Period 1 DI</th>
<th>Period 2 DI</th>
<th>Period 1 CAP</th>
<th>Period 2 CAP</th>
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<td>BT</td>
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<td>67.0 ± 1.7 A</td>
<td>55.7 ± 1.5 A</td>
</tr>
<tr>
<td>Water Intake</td>
<td>150.0 ± 8.9 A</td>
<td>113.6 ± 9.4 A</td>
<td>156.6 ± 12.9 A</td>
<td>116.3 ± 9.2 A</td>
</tr>
<tr>
<td>Urine Output</td>
<td>122.1 ± 9.4 A</td>
<td>87.4 ± 7.1 A</td>
<td>128.5 ± 12.8 A</td>
<td>91.5 ± 9.4 A</td>
</tr>
<tr>
<td>Tidal Volume</td>
<td>1.78 ± 0.22 A</td>
<td>1.73 ± 0.10 A</td>
<td>1.98 ± 0.21 A</td>
<td>1.61 ± 0.07 A</td>
</tr>
<tr>
<td>Frequency</td>
<td>157.0 ± 3.0 A</td>
<td>146.0 ± 6.0 A</td>
<td>153.0 ± 3.0 A</td>
<td>134.0 ± 4.0 B</td>
</tr>
</tbody>
</table>

Bodyweight Corrected Oxygen Consumption ([(ml/min) x 1000]/(g)), Body Temperature (BT, °C), Food Intake (g/24/Kg), Water Intake (ml/24/Kg), Urine Output (ml/24/Kg), Bodyweight Corrected Tidal Volume [(ml x 1000)/g] and Frequency of Breathing (bpm) of SHHF rats given control (DI, n=18) and captopril (CAP, n=15) treatments.

Bodyweight Corrected Tidal Volume and Frequency of Breathing data in each subset was recorded when the rats were 12-13 weeks of age (Period 1) and then again at 21-22 weeks of age (Period 2). The DI group contained 18 test subjects and CAP group had 15 animals. Oxygen Consumption and Body temperature data in each subset was recorded when the rats were 12-13 weeks of age and then again at 21-22 weeks of age. The metabolic factors were recorded the subsequent week. Different letters denote significant differences between each group's treatment and time. Lower food and water intake and urine outputs are not the results of treatment. Values are Means ± the Standard Error.
Table 2. Body Weights and Metabolic Cage Data from the 2\textsuperscript{nd} Study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight</th>
<th>Fluid Intake</th>
<th>Food Intake</th>
<th>Urine Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI-DI 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 M2</td>
<td>636.7 ± 8.7</td>
<td>119.4 ± 13.7</td>
<td>55.1 ± 1.34</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 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 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 ± SEM. The M2 refers to the second time period and M3 the 3\textsuperscript{rd} time period when metabolic measurements were made. DI -DI (n=9) refers to rats who received deionized (DI) water at both time points. DI-ARG (n=11) refers to rats who received DI water, but who then received l-arginine in M3. Cap-DI (n=9 for each group) both received captopril in M2 and DI in M3. CAP-CAP refers to rats that received captopril in M2 and in M3. Units for Body weight are in grams, for fluid intake and urine output are ml/24hr/Kg and for food intake are g/24/hr/Kg. The asterisks indicate significant effects (P<0.02 to P=0.0001) of period 3 relative to period 2 treatments. Note that only rats receiving captopril at both time points exhibited no change in fluid intake, food intake or in urine output from period 2 to 3.
Table 3. Body Weight-Corrected Tidal Volume and Frequency in Study 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tidal Volume</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI-DI 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 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 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 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 ± SEM. The R2 refers to the second time period and R3 the 3rd time period when respiratory measurements were made. DI-DI (n=9) refers to rats who received deionized (DI) water at both time points. DI-ARG (n=11) refers to rats who received DI water, but who then received l-arginine in R3. CAP-DI rats received captopril in R2 and DI during period 3. CAP-CAP (n=9 for each group) both received captopril at both times. Units are Bodyweight Corrected Tidal Volume [(ml x 1000)/g] and Frequency of Breathing (bpm). The asterisks indicate significant effects of treatments from period 2 to period 3. Breathing frequencies of rats receiving or who had received captopril were significantly less (**, P<0.01) than rats with a prior history or DI or who received arginine.
Figure 1.
Figure 2. Bodyweight Corrected Minute Ventilation

Figure 2.
Figure 3
Figure 4.
Figure 5.
Breathing Patterns in SHHF Obese Rats

A

B

C

Normal Pattern

Figure 6.