Exercise Intolerance in Rats With Hypertensive Heart Disease Is Associated With Impaired Diastolic Relaxation

Marco Guazzi, Daniel A. Brenner, Carl S. Apstein, Kurt W. Saupe

Abstract—A decrease in functional capacity is one of the most important clinical manifestations of hypertensive heart disease, but its cause is poorly understood. Our purpose was to evaluate potential causes of hypertension-induced exercise intolerance, focusing on identifying the type(s) of cardiac dysfunction associated with the first signs of exercise intolerance during the course of hypertensive heart disease. Exercise capacity was measured weekly in Dahl salt-sensitive rats as they developed hypertension as well as in Dahl salt-resistant control rats. Exercise capacity was unchanged from baseline during the first 8 weeks of hypertension, suggesting that hypertension itself did not cause exercise intolerance. After 9 to 12 weeks of hypertension, exercise capacity decreased in salt-sensitive rats but not in control rats. After 10 weeks of hypertension, indices of diastolic function (early truncation of the E wave), as assessed by echocardiography at rest, were decreased in the salt-sensitive rats. When exercise capacity had decreased by ≈25% in a rat, the heart was isolated, and left ventricular (LV) compliance and systolic function were measured. At that time point, LV hypertrophy was modest (an ≈20% increase in LV mass), and systolic function was normal or supernormal, indicating that exercise intolerance began during “compensated” LV hypertrophy. Passive LV compliance remained normal in salt-sensitive rats. Thus, in this model of hypertensive heart disease, exercise intolerance develops during the compensated stage of LV hypertrophy and appears to be due to changes in diastolic rather than systolic function. However, studies in which LV function is assessed during exercise are needed to conclusively define the roles of systolic and diastolic dysfunction in causing exercise intolerance. (Hypertension. 2001;37:204-208.)

Key Words: exercise • hypertension, experimental • hypertrophy • echocardiography • heart • rats

One of the most important clinical manifestations of hypertensive heart disease is a decrease in functional capacity (exercise intolerance), in that functional capacity is a major determinant of quality of life, and changes in functional capacity provide important prognostic information.1 Despite this importance and the fact that a decrease in functional capacity occurs even in patients with mild hypertension, the cause of exercise intolerance in hypertensive individuals is poorly understood.2 Measures of resting systolic cardiac function, such as ejection fraction, are generally poor predictors of exercise capacity.3 Observations in patients and animal models of heart disease suggest that left ventricular (LV) diastolic dysfunction is often a prime contributor to impaired exercise capacity.4 Isolated diastolic dysfunction is a well-described clinical syndrome that often results in the clinical presentation of congestive heart failure in patients with normal systolic function.5 Reports investigating the relationship between development of isolated diastolic dysfunction and exercise performance are lacking. Likewise, information on how exercise capacity changes during the progression from normotension to hypertension to LV hypertrophy (LVH) is limited, as is our understanding of the causes of the exercise intolerance. This lack of understanding exists in part because exercise capacity has not been measured in longitudinal studies of patients or animal models of hypertension.

Accordingly, the present study was undertaken to determine when during the natural history of hypertensive heart disease exercise intolerance develops and to evaluate potential causes of the decreased exercise capacity. We were particularly interested in identifying the type(s) of cardiac dysfunction associated with the first signs of exercise intolerance. The Dahl salt-sensitive (SS) rat model was used because of its well-characterized progression from normotension to hypertension to LVH and heart failure during ≈3 to 4 months on a high-salt diet.6,7 To evaluate potential causes of exercise intolerance, M-mode and mitral Doppler echocardiography were measured in vivo in SS as well as in normotensive salt-resistant (SR) rats, with subsequent study of the isolated hearts.

Methods

Animal Model
Male inbred Dahl SR (n=17) and SS (n=19) rats were obtained from Harlan Sprague Dawley, Indianapolis, Ind. All procedures were
approved by the Boston University School of Medicine Animal Care and Use Committee. Rats were fed according to the protocol described by Inoko et al7 with the following modifications: The rats arrived at 8 to 9 weeks of age and were fed a low-salt diet (0.12% NaCl) during a 2-week acclimatization period. At the end of this period, all rats were fed a high-salt diet (7.8% NaCl).

Measurement of Systolic Pressure and Exercise Capacity
During the 2-week acclimatization period, arterial systolic blood pressure (SBP) was measured during 3 sessions in each rat by use of the tail-cuff method. A minimum of 3 measurements of systolic pressure was made in each animal during each session; each measurement was conducted in a quiet warm (30°C) room.8 Once the high-salt diet was initiated, SBP was measured in triplicate weekly in each rat.

To measure maximal exercise capacity (time to exhaustion during a standardized exercise protocol), rats were first familiarized with a motor-driven treadmill (Columbus Instruments) during the 2-week acclimatization period. During the first week of acclimatization, this consisted of 3 sessions of slow walking on the treadmill for 10 to 15 minutes each. During the second week of acclimatization, rats underwent 3 maximal exercise capacity tests, with the final 2 averaged to give the pre–high-salt diet (baseline) value for maximal exercise capacity. Each exercise test was performed after at least 1 day of rest. Once the high-salt diet was initiated, exercise capacity was measured weekly in each rat. When it had decreased by ~25% from baseline in a given SS rat, that rat was euthanized, and the isolated heart was studied. For each SS rat euthanized in this manner, an SR rat was also chosen for euthanasia and study. These terminal studies were conducted 9 to 12 weeks after the start of the high-salt diet.

The protocol for evaluating exercise capacity consisted of 15 minutes at 20 meters/min, with 5-meter/min increases in speed every 5 minutes. Treadmill incline was 7° throughout. Rats were exercised until exhaustion, defined as the inability to continue running despite contact with a shock bar located at the rear of the treadmill belt.

In Vivo LV Function Evaluation by Echocardiography
Echocardiograms were recorded in a subset of SR (n = 10) and SS (n = 10) rats 10 weeks after the start of the high-salt diet, during light ketamine/xylazine (35 and 5 mg/kg, respectively) anesthesia. All studies were performed with a 7.5-MHz short focus transducer with a 60-Hz acquisition rate (Hewlett-Packard Sonos 5500). Images were obtained in the parasternal long-axis and short-axis and apical 2-chamber and 4-chamber views. Real-time images were stored on videotape for subsequent offline analysis. Echocardiographic studies were analyzed independently by 2 experienced readers, and their values were averaged. M-mode recordings were obtained through the septal and posterior walls. LV end-diastolic diameter (EDD) and end-systolic diameter (ESD) and septal and posterior wall thicknesses were measured leading edge to leading edge, according to the American Society of Echocardiography–recommended guidelines.9

The endocardial fiber fractional shortening (%Fs) was measured according to the following formula: %Fs = [(EDD – ESD)/EDD]100. LV mass was calculated by using a standard formula, which assumes a spherical LV geometry: LV mass = 1.04[(EDD + PWT + SWT)1/3 – EDD1], where PWT is posterior wall thickness, and SWT is septal wall thickness. Pulsed-wave Doppler spectra of mitral inflow were recorded from an apical 4-chamber view, with the sample volume placed at the tips of the mitral leaflets in diastole. The following measurements were carried out: mitral peak flow velocity of early filling wave (E) and peak flow velocity of late filling wave (A), their ratio (E/A), the E-wave deceleration time (Edec time), the E-wave deceleration slope (Edec slope), and the isovolumic relaxation time, defined as the time between the aortic flow termination and the beginning of mitral flow.

Isolated Perfused Heart Studies
To better characterize the hypertension-induced changes in LV function, isolated whole heart studies were performed on each heart by using an isolated isovolumically beating (balloon-in-LV) heart preparation perfused with red blood cells as previously described.10 Briefly, a fluid-filled balloon connected to a Statham P23Db pressure transducer was placed into the LV through an incision in the left atrium. The hearts were submerged in a 37°C saline bath. Coronary perfusion pressure was set at 90 mm Hg for all hearts. Hearts were paced at 5 Hz (model 59, Grass Instruments) by use of epicardial electrodes. The coronary perfusate consisted of bovine red blood cells at a hematocrit of 0.40, which were washed and resuspended in Krebs-Henseleit buffer as follows (mmol/L): NaCl 118, KCl 4.7, CaCl2 2.0, KH2PO4 1.2, MgSO4 1.2, NaHCO3 26.6, glucose 5.5, lactate 1.0, and palmitic acid 0.4, along with 40 g/L BSA. The perfusate was equilibrated with 20% O2, 3% CO2, and 77% N2 to achieve a PO2 of 120 to 140 mm Hg and a pH of 7.4.

LV function and compliance in the isolated hearts were assessed by measuring systolic and end-diastolic pressures as LV balloon volume was increased in 0.05-ML increments until an end-diastolic pressure of 40 mm Hg was reached.

Statistical Analysis
The data presented are mean±SE unless otherwise specified. Comparison of groups was performed by the unpaired Student t test. Where an analysis for multiple measures was necessary, ANOVA was performed. A value of P<0.05 was considered significant.

Results

Animal and Cardiac Characteristics
Body weight measured at the time of euthanasia was not significantly different between the 2 groups (380±6 g in SR, 385±6 g in SS), nor was tibia length (3.88±0.04 cm in SR, 3.91±0.04 cm in SS). LVH was present in the SS group, as indicated by a significantly (P<0.05) increased whole heart weight (1.40±0.05 g in SR and 1.62±0.04 g in SS) and ratio of heart weight to tibia length (0.36±0.01 g/cm in SR and 0.41±0.01 g/cm in SS). The lung wet weight–to–dry weight ratio, an index of pulmonary congestion, was not different between groups.

SBP measured in vivo before the high-salt diet (time 0 in Figure 1) was not different between groups. After 2 weeks of a high-salt diet, SBP was significantly elevated in the SS rats. This hypertension persisted throughout the remaining experimental period, as shown in Figure 1.

Maximal Exercise Capacity
Maximal exercise capacity (minutes to exhaustion) at baseline averaged 31±1 minutes for SR rats and 25±1 minutes for SS rats (P<0.05). Longitudinal changes in maximal exercise capacity are shown in Figure 1. In SR rats, exercise capacity remained within 10% of baseline throughout the 12 weeks. In SS rats, exercise capacity did not change from baseline during the first 8 weeks of high-salt diet/hypertension. However, between weeks 9 and 12, exercise capacity decreased in each SS rat. Exercise capacity had decreased to 65±5% of baseline in the SS rats at their final exercise test.

In Vivo Assessment of LV Function
In a subgroup of animals (10 SR and 10 SS rats), 2D, M-mode, and mitral Doppler echocardiography were used to assess LV morphometry and function within 24 hours of the maximal exercise capacity test during week 10. As shown in
the Table, septal and posterior wall thicknesses were significantly larger in the SS compared with SR rats. From these values, calculation of LV mass indicated that SS hearts were 0.2 g larger than SR hearts. This is in good agreement with the difference in whole heart weights (see above) and indicates that all of the increase in whole heart weight in the SS hearts was localized in the LV. M-mode echocardiography indicated that LV EDD was similar in the 2 groups, whereas SS rats had a somewhat decreased end-systolic volume \((P=0.06)\), indicating an increase in fractional shortening (Table). Heart rate measured during the echocardiograms was slightly, but significantly, higher in the SS compared with SR hearts (254±8 and 220±10 bpm, respectively; \(P<0.05\)). Taken together, these data suggest that SS hearts were in the compensated LVH stage of the natural history of hypertensive heart disease.

The pattern of flow through the mitral valve differed in potentially important ways in the 2 groups of hearts. Although the E-wave and A-wave amplitudes and their ratio were not different between groups, the deceleration phase of the E wave was significantly shorter and had a significantly higher slope in the SS hearts (Table, Figure 2). This pattern of a truncated E wave but normal E/A ratio constitutes “pseudonormalization.”

**LV Function in Isolated Hearts**

The effects of increasing LV volume on end-diastolic and systolic pressures are shown in Figure 3. The end-diastolic pressure-volume curves were indistinguishable between SR and SS rats, indicating that despite the increase in LV mass in the SS hearts, there was no measurable difference in passive LV compliance between the 2 groups. The systolic pressure-volume curve was shifted upward in the SS hearts, consistent with compensated LVH.

**Discussion**

In patients, even mild hypertension is associated with a decreased exercise capacity, but mechanism(s) underlying this exercise intolerance are poorly understood. In studying salt-sensitive rats longitudinally, we found that 8 weeks of hypertension did not alter exercise capacity. This is consistent with the finding that after 8 weeks of aortic constriction, exercise capacity was undiminished in rats. After 9 to 12 weeks of hypertension, we observed that exercise intolerance did develop. At that time in the natural history of hypertension, significant LVH had occurred, and systolic function was normal or supernormal, suggesting that exercise intolerance began to occur during the “compensatory” stage of LVH.
Evaluation of diastolic function in the hypertensive rats indicated that passive LV compliance was normal but that LV relaxation was impaired. Although ischemia can cause impaired LV relaxation, it is unlikely to have been a factor in this model because the diastolic dysfunction occurred at rest, and SS rats have improved ischemic tolerance at this stage of LVH.

Diastolic function is considered normal when the LV can fill to an appropriate volume without an elevated filling pressure. To accomplish this, the myofilaments must rapidly release tension at the end of systole, and once tension is released, the passive properties of the LV must allow LV volume to increase normally without an excessive increase in LV filling pressure. The pattern of mitral flow observed in the SS hearts, a truncated E wave, is consistent with a rapid increase in LV pressure during early diastole, resulting in equilibration of pressure between the left atrium and ventricle so that mitral inflow ceases early. Therefore, the truncation of the E wave suggests a loss of early diastolic distensibility secondary to impaired relaxation, with or without a loss of elastic recoil. In patients, a truncated early mitral flow with a normal E/A ratio constitutes the pseudonormalized pattern.

The pattern of impaired early diastolic filling that we observed in the SS rats is similar to that reported in rat hearts with LVH secondary to aortic banding. In that study, similar to our findings in hypertensive LVH, Dahl SS rats with LVH have a normal LV compliance consistent with a study by Morii et al and indicates that abnormalities in LV filling early during diastole can precede, and are therefore not necessarily caused by, changes in passive LV compliance.

The fact that exercise capacity is sensitive to changes in diastolic function is not surprising because even in normal healthy humans, resting diastolic function is thought to be an important determinant of exercise capacity. This relationship between diastolic function and exercise tolerance is clinically useful in that exercise intolerance is frequently the first manifestation of diastolic heart failure. Similar to our finding in hypertensive heart disease, Sumimoto et al have demonstrated that decreased exercise capacity after myocardial infarction is likely related to diastolic dysfunction. This sensitivity of exercise capacity to diastolic function is likely due to the fact that as heart rate increases during exercise, the period of LV filling shortens simultaneously with an increase in cardiac output, necessitating a large increase in flow rate through the mitral valve. If LV diastolic function is impaired, either an inadequate volume of blood will enter the LV during diastole, or the normal volume will enter, but at a high filling pressure. Furthermore, diastolic dysfunction can result in impaired systolic function, as elegantly demonstrated by Cuocolo et al. These investigators showed that poor diastolic filling during exercise can lead to a low end-diastolic volume, which not only lowered stroke volume but also decreased systolic function secondary to a decreased preload. In patients with hypertensive heart disease, abnormal LV filling occurs early in the natural history of mild to moderate hypertension, but a link between this altered diastolic filling and a decrease in exercise capacity has not previously been firmly established.

Determining the mechanism by which hypertension causes exercise intolerance is complicated by the difficulty in measuring and interpreting data collected during maximal exercise. For example, in a patient with exercise intolerance, a low rate of early mitral flow during maximal exercise could be either the cause or result of the low maximal cardiac output that would be expected in an exercise-intolerant patient. This issue is less problematic in studies in which LV function is assessed at rest, inasmuch as intergroup differences in resting cardiac output would be comparatively small. Changes in skeletal muscle biochemistry secondary to LVH have been suggested as a possible contributor to exercise intolerance. Because the changes in skeletal muscle biochemistry are subtle (when present at all) even in hearts with twice the degree of LVH as found in our SS hearts, it seems unlikely that these changes played an important role in causing exercise intolerance in the present study.

Several limitations of the present study merit mention. First, even before initiation of the high-salt diet, SS rats had a significantly lower maximal exercise capacity than did SR rats; this finding was likely due to the slight genetic differences in the 2 strains. Thus, maximal exercise capacity data in the SR rats are presented to establish that the eventual decline in maximal exercise capacity in the SS rats was secondary to their hypertension. A second limitation was that many of the variables measured with echocardiography are known to be influenced by preload, heart rate, and SBP. Because HR and SBP were not matched in the 2 groups and because preload...
was not measured, this must be considered as a possible reason for the intergroup differences in systolic and diastolic function. It should also be noted that echocardiograms were recorded only at rest; thus, it is not known whether SS rats demonstrated impaired diastolic function during maximal exercise. However, it is reasonable to expect that if diastolic dysfunction is present under the relatively unstressed hemodynamic conditions present during general anesthesia, it would also be present and probably magnified during maximal exercise. A further limitation of the present study is that the timing and underlying cause of exercise intolerance during the natural history of hypertensive heart disease is likely model dependent; thus, the generalizability of our findings to other causes of hypertensive heart disease is unknown and remains to be tested.

In summary, we found in a rat model of SS hypertension that during the natural history of hypertension, exercise intolerance does not develop until the compensated stage of LVH occurs, indicating that the hypertension itself does not cause exercise intolerance. The exercise intolerance occurred at a time of normal or supernormal systolic function and was associated with abnormalities in resting diastolic function. Interestingly, it was not a change in passive compliance of the LV but abnormalities of relaxation during early diastole that were associated with exercise intolerance. These data support the fundamental role of diastolic function in determining maximal exercise capacity, but further studies assessing LV function during maximal or near-maximal exercise are required to more firmly define the relative roles of systolic and diastolic dysfunction in the cause of exercise intolerance.

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References

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