Diastolic Compliance Is Reduced in Obese Rabbits
Joan F. Carroll, Richard L. Summers, David J. Dzielak, Kathy Cockrell, Jean-Pierre Montani, H. Leland Mizelle

Abstract—Obesity often leads to symptoms of cardiopulmonary congestion associated with normal systolic but abnormal diastolic function. This study analyzed alterations in passive diastolic compliance in obesity using the rabbit model. New Zealand White rabbits were fed a normal (n = 8) or 10% added fat diet (n = 8). After 12 weeks, rabbits fed the high fat diet developed obesity (5.34 ± 0.11 versus 3.68 ± 0.04 kg, P ≤ 0.05) and left ventricular hypertrophy (1.37 ± 0.07 versus 0.98 ± 0.03 g dry weight, P ≤ 0.05). Compliance was assessed with the isolated heart preparation by analyzing the passive end-diastolic left ventricular pressure-volume relationship. The pressure-volume relation was fit to an exponential function by regression analysis; results showed that the modulus of stiffness was greater in obese than in lean rabbits (1.21 ± 0.16 versus 0.83 ± 0.05, P ≤ 0.05), indicating that diastolic compliance was reduced. Computer simulation analyses suggested that an isolated reduction in diastolic compliance may contribute to elevated cardiac filling pressures and exercise intolerance. These data suggest that diastolic compliance is reduced early in the development of obesity and may be an important component in the reduction of cardiac reserve in obesity. (Hypertension. 1999;33:811-815.)

Key Words: heart □ stroke volume □ obesity □ computer modeling □ diastolic pressure-volume relationship

Obesity is independently associated with an increase in morbidity and mortality, resulting primarily from increased incidence of cardiovascular diseases such as stroke, coronary artery disease, and congestive heart failure.1 The precise mechanisms leading to greater risk for developing congestive heart failure in obesity are unknown but may stem from the increased work load on the heart imposed by the combination of increased cardiac output, increased intravascular volume, hypertension, and cardiac hypertrophy.2 Interestingly, some obese patients with symptoms of systemic and pulmonary congestion present with normal systolic function; however, diastolic function is often abnormal.3 In fact, it has been estimated that 35% to 40% of patients with congestive heart failure present with diastolic dysfunction only.4

Diastolic dysfunction may be simply described as a condition in which diastolic filling is impeded. However, diastolic filling of the left ventricle is a complex process determined by the interaction of several factors, including active ventricular relaxation and passive properties influencing left ventricular compliance. Active relaxation is related to calcium reuptake by the sarcoplasmic reticulum, whereas passive ventricular filling properties are determined by ventricular wall thickness, the dimensions of the ventricular cavities, and the structural properties of the cardiac tissue itself. A few studies have attempted to examine the diastolic pressure-volume relationship,5,6 and the data suggested that morbidity obese patients exhibit abnormalities in diastolic filling, with or without the presence of hypertension or cardiac hypertrophy.7,8

Although there is evidence that diastolic filling is altered in obesity,7 it is difficult to determine from clinical studies what role altered diastolic compliance plays in diastolic dysfunction. Little is known about the mechanisms underlying abnormal diastolic function in obesity or the relative contributions of decrements in active versus passive relaxation to filling abnormalities. In addition, little is known about how quickly diastolic dysfunction becomes manifest during the development of obesity. Therefore, our purpose was to examine the passive end-diastolic pressure-volume relationship in isolated hearts of obese and control rabbits to determine the presence and extent of obesity-related decrements in passive diastolic compliance. In addition, we used computer models of circulatory function to predict systemic effects resulting from reduced compliance both at rest and during exercise.

Methods

Animals
Experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. All animal care and use programs were carried out according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86-23, revised 1985) and regulations of the Animal Welfare Act. Female New Zealand White rabbits were purchased when they were approx-
immediately 15 to 17 weeks old and weighing 3.25 to 3.75 kg (Myrtle’s Rabbity, Thompson Station, Tenn.). They were housed individually in a humidity- and temperature-controlled room with a 12-hour light cycle and were fed 100 to 120 g/d of standard rabbit chow (Purina Mills). After a 2-week acclimation period, they were randomly divided into 2 groups, one designated to remain lean (n=8) and the other to become obese (n=8). The lean group continued with the same dietary regimen, which is an appropriate maintenance diet for a normal nonlactating adult rabbit.6 The obese group was given a high fat diet, ad libitum, which consisted of standard rabbit chow with 10% added fat. The excess fat in the diet was two thirds corn oil and one third lard. Experiments were performed after rabbits had been on their respective diets for 12 weeks.

**Blood Pressure Measurement and Surgical Procedures**

Acute blood pressure measurement and surgical procedures for heart excision have been described previously.10 Briefly, blood pressure in the conscious, unanesthetized rabbit was measured directly from a central ear artery catheter for 60 to 90 minutes. A small arterial blood sample was then withdrawn for measurement of hematocrit and plasma protein. After blood sampling, general anesthesia was induced with 4% to 5% isoflurane with an oxygen flow of 1 L/min administered via a face mask. The heart was then subjected to cardioplegic arrest and prepared for the Langendorff preparation. First, venous return was stopped by tying sutures around the superior and inferior vena cavae. Pressure was then placed on an aortic ligature, and the heart was arrested by infusing cold (4°C) Euro-Collins solution retrograde into the aortic root. The mitral valve was excised, and a latex balloon containing a high-fidelity micromanometer was positioned in the left ventricle for pressure measurements.

**Isolated Heart Protocol**

The relationship between left ventricular end-diastolic pressure and volume was determined with the isovolumic Langendorf-isolated heart preparation as previously described.10 Briefly, signals from the high-fidelity micromanometer within the latex balloon were sampled 6 times each minute for 6 seconds each to detect peak pressure, end-diastolic pressure, peak +dP/dt, and peak –dP/dt for each burst. Heart function was allowed to stabilize for approximately 30 minutes, after which control measurements of peak pressure, end-diastolic pressure, and peak +dP/dt and –dP/dt were recorded. Coronary flow was also determined by a timed measurement of coronary effluent. After stabilization, 0.3 mL fluid was added to the latex balloon every 3 minutes until the end-diastolic pressure was approximately 20 mm Hg. Reported values were the average of measurements taken during the final 40 seconds of each 3-minute period when function had stabilized.

**Analytic Methods**

Hematocrit was measured in duplicate with a microhematocrit centrifuge (Adams Autocrit Centrifuge, Clay Adams) and a micro-capillary tube reader. Plasma protein was measured by refractometry.

**Statistical and Computer Simulation Analyses**

Descriptive data comparing lean and obese rabbits included body weight, blood pressure, heart rate, left and right dry ventricular weights, and plasma hematocrit and protein. These variables were analyzed by unpaired t tests, with significance accepted at the 0.05 level.

Compliance was assessed by analyzing the passive end-diastolic left ventricular pressure-volume relationship. To enable comparisons among hearts, the volume at which end-diastolic pressure equaled zero was designated as the zero volume in each heart. Pressure data at increments of 0.3 mL up to a volume of 1.5 mL were then analyzed with a repeated-measures multivariate analysis of variance. When there was a significant group × volume interaction, pressure data at each volume were analyzed using two-sample t tests with a Bonferroni correction (P<0.01). Similar analyses were performed for peak +dP/dt and –dP/dt values. In a second analysis, the pressure-volume relation was fit to an exponential function by regression analysis.11 Volumes were normalized to left ventricular weight in order to incorporate both heart size and muscle thickness and minimize their influence. From the derived equations, the average modulus of stiffness was compared between lean and obese rabbits with an unpaired t test. Significance was accepted at the 0.05 level. All data are expressed as mean±SEM.

It is difficult to interpret isolated organ findings in the context of the whole animal, so experimental results were also examined by systems analysis using a computer model of circulatory function. We used a derivative of the well-established circulatory model of Guyton and colleagues12 and a previously published model of cardiac diastolic dynamics.13 This model uses known physiological parameter interrelationships in continuous feedback and feedback loops to predict values of a variety of hemodynamic variables over a wide range of perturbations. In this model, cardiac output is determined from factors that influence venous return and the cardiac Starling curve, with diastolic compliance (both active and passive) contributing to resistance to cardiac filling. Within this framework, changes in passive diastolic compliance seen experimentally were incorporated into the model, and the theoretical effects on cardiac output, urinary output, atrial pressure, and extracellular fluid volume were predicted. The effect of increased stress on a heart with reduced compliance was also simulated in an exercise experiment, in which exercise was simulated by a general sympathetic discharge with increases in cardiac strength and vascular tone. Cardiac output and right atrial pressure values during simulated exercise were compared in models with and without compliance changes.

**Results**

After 12 weeks of a high fat diet, body weight of obese rabbits increased 53%, from an initial average of 3.48±0.11 to 5.34±0.11 kg. In comparison, lean rabbits averaged 3.49±0.03 kg initially and 3.68±0.04 kg after 12 weeks. The difference between lean and obese rabbits after 12 weeks was statistically significant (P<0.05). Obesity was associated with resting tachycardia and mild hypertension. Resting heart rates were 19% higher in obese than lean rabbits (243±12 versus 204±12 bpm, P<0.05), and mean arterial pressure measurements averaged 96±2 mm Hg in obese rabbits compared with 90±2 mm Hg in lean rabbits (P=0.08). Although hypertension in this experiment was modest, in other experiments using this model we have demonstrated increases in blood pressure averaging 10% to 21%,10,14–16

Obese rabbits also developed cardiac hypertrophy. Dry left ventricular weights were 40% heavier in obese than lean rabbits (1.37±0.08 versus 0.98±0.03 g, P<0.05), and dry right ventricular weights were 57% heavier in obese rabbits (0.66±0.06 versus 0.42±0.03 g, P<0.05). Hematocrit measurements were higher in obese rabbits (0.41±0.01 versus 0.36±0.01, P<0.05), as were plasma protein levels (5.6±0.1 versus 5.4±0.1 g/dL, P<0.05).

Figure 1A illustrates the end-diastolic pressure-volume relationship when data were calculated to yield a volume of zero when end-diastolic pressure was zero. The mean volume at which end-diastolic pressure equaled zero did not differ between lean and obese hearts (0.73±0.02 versus 0.73±0.02 mL, respectively; P>0.05). However, there was a significant group × volume interaction (P<0.05). Comparison of lean and obese hearts indicated significant pressure differences at 0.9 mL (4.8±0.4 versus 9.1±1.3 mm Hg, respectively), 1.2 mL (6.0±0.4 versus 12.3±2.0 mm Hg, respectively), and 1.5 mL (7.4±0.6 versus 13.8±2.7, respectively) (all P<0.01).
Figure 1B illustrates the pressure-volume relationship in isolated hearts of lean and obese rabbits. A, Relationship obtained when volume is calculated to equal zero when EDP = 0. B, Relationship obtained when volume is normalized for left ventricular weight and the pressure-volume relationship fit to the exponential relationship $P = b e^{k c V}$ (where $P =$ left ventricular end-diastolic pressure, $V =$ left ventricular end-diastolic volume, $k_c =$ modulus of chamber stiffness, and $b =$ curve fitting parameter).

Figure 1. End-diastolic pressure (EDP)–volume relationships in isolated hearts of lean and obese rabbits. A, Relationship obtained when volume is calculated to equal zero when EDP = 0. B, Relationship obtained when volume is normalized for left ventricular weight and the pressure-volume relationship fit to the exponential relationship $P = b e^{k c V}$ (where $P =$ left ventricular end-diastolic pressure, $V =$ left ventricular end-diastolic volume, $k_c =$ modulus of chamber stiffness, and $b =$ curve fitting parameter).

Computer Simulation Analyses

In computer simulations, we determined theoretical systemic effects associated with the in vitro reduction in compliance. Figure 2A illustrates the effect of reduced compliance in the resting state. Initial decreases were seen in cardiac output and urinary output, whereas right atrial pressure and extracellular fluid volume increased. In the long term, cardiac output returned toward normal at the cost of further fluid retention and higher cardiac filling pressure. Figure 2B illustrates the effect of decreased diastolic compliance in a model made to simulate moderate exercise. Compared with a model without compliance changes, overall cardiac output and systemic flow were reduced during exercise, despite further increases in filling pressure. Although this result has been seen previously in live obese animals, these in vitro and computer simulation studies suggest that reduced diastolic compliance may be a major factor in the abnormal hemodynamic response to exercise in obesity.

Discussion

We have demonstrated that diastolic compliance is reduced in isolated hearts of obese rabbits. This is associated with several cardiovascular abnormalities, including hypertension, resting tachycardia, and cardiac hypertrophy. Reduced com-

Figure 2. Computer models of circulatory function. A, Acute and chronic effects of a decrease in diastolic compliance on resting cardiac output (CO), urinary output (UO), right atrial pressure (RAP), and extracellular fluid volume (ECFV). B, Circulatory effect of decreased diastolic compliance on cardiac output and right atrial pressure during light exercise under control conditions and with a decrease in diastolic compliance.
compliance in obesity was not associated with alterations in the peak rate of either pressure rise or pressure decline. In conjunction with isolated heart experiments, our computer simulations suggest that a reduction in compliance alone may produce significant in vivo hemodynamic consequences, including increases in atrial pressure and volume overload. Although obesity is associated with increases in morbidity and mortality from cardiovascular diseases such as congestive heart failure,1 we believe that these are the first data to clearly show early development of reduced diastolic compliance in an experimental model of obesity.

The hypertension observed in this study was modest, averaging 6 mm Hg higher (7%) in obese than lean rabbits; the increase in resting heart rate in obese rabbits averaged 19%. Although the degree of resting tachycardia in the present study was similar to that in earlier studies using this model, we have previously measured greater obesity-related increases in blood pressure (eg, 10% to 21%).10,14–16 Still, we believe that this is an underestimation of the chronic elevations in blood pressure and heart rate in obese rabbits. We have preliminary data demonstrating that blood pressure and heart rate measured 18 h/d with telemetry averaged 24% and 38% higher, respectively, in obese than lean rabbits (n=4 each group).

The effects of obesity on heart function in humans are often studied noninvasively with echocardiography.8,18 However, noninvasive methods such as echocardiography are limited in the extent to which mechanisms underlying these abnormalities can be identified. Animal models of obesity also have yielded little concrete information on the potential role of obesity in altering diastolic compliance, because of either contradictory results19,20 or use of an animal model with many characteristics that are clearly different from human obesity.19–21

The rabbit model has been shown to be useful in the study of obesity-related hypertrophy. We have demonstrated that obesity in this model was associated with ventricular hypertrophy that was mainly due to myocyte hypertrophy and not fatty infiltration.14 Ventricular hypertrophy is still evident when dry ventricular weight is normalized for lean body mass. Using data from Dwyer et al,22 we estimated lean body mass of animals in the present experiment and calculated ratios of dry left ventricular weight to lean body mass of 0.29±0.01 and 0.39±0.01 for lean and obese rabbits, respectively (P≤0.05). We also used echocardiography to study the geometric nature of obesity-related hypertrophy and demonstrated that there was a combined concentric and eccentric remodeling, with increases in both wall thickness and chamber diameter. Functionally, obese rabbits showed an increase in peak atrial flow velocity (A wave) and an increase in the ratio of peak atrial to peak early flow velocity (A/E).16 Although early diastolic filling rate (E wave) did not differ between lean and obese rabbits, there was a slightly larger stroke volume in obese rabbits, necessitating a compensatory increase in the contribution of atrial filling to achieve an adequate end-diastolic volume.16 In addition to altered rates of filling late in diastole, we have demonstrated in the present study that reduced passive diastolic compliance is another important factor in obesity-related abnormalities of diastolic filling.

We noted in the present investigation that peak +dP/dt and –dP/dt values did not differ significantly between lean and obese hearts. This is not incompatible with a reduction in compliance because the rate and extent of relaxation are two different properties of the myocardium. Rate of relaxation, as illustrated by –dP/dt, affects the pressure-volume relation early in diastole because it affects the atrioventricular pressure gradient and ventricular filling during the rapid phase of filling.23 In agreement with these data, we have found echocardiographic evidence that early diastolic filling is not altered in obesity.16 In contrast, the extent of relaxation is the state of the myocardium after relaxation has been completed, and this determines equilibrium length and volume of the left ventricle. If the extent of relaxation is impaired, as the present experiment suggests, equilibrium volumes will be smaller and end-diastolic pressures higher.

Ventricular hypertrophy may affect the extent of relaxation because the associated decrease in capillary density and coronary vasodilator reserve results in ischemia.23 Although coronary flow was not significantly different between lean and obese hearts in the present study, it did appear somewhat reduced in obese hearts and may have contributed to reduced compliance. Hypertrophy may also reduce uptake of calcium by the sarcoplasmic reticulum. In addition to slowing the rate of relaxation, this may affect the extent of relaxation if greater amounts of calcium remain in the cytoplasm at end-relaxation. Future work is needed to determine whether function of the sarcoplasmic reticulum is reduced in obesity.

Another contributing factor to decreased diastolic compliance in obesity may be collagen deposition. We have shown that interstitial and perivascular collagen is significantly increased in obese rabbit hearts.24 Although this has been identified as a factor in increased diastolic stiffness in renovascular hypertension and pressure-overload models of hypertension, increased concentrations of collagen have not been identified in obesity. The initiating event in myocardial fibrosis is not clear but may be associated with neural or humoral factors such as the renin-angiotensin system.25 Although coronary flow was not significantly different between lean and obese hearts in the present study, it did appear somewhat reduced in obese hearts and may have contributed to reduced compliance. Hypertrophy may also reduce uptake of calcium by the sarcoplasmic reticulum. In addition to slowing the rate of relaxation, this may affect the extent of relaxation if greater amounts of calcium remain in the cytoplasm at end-relaxation. Future work is needed to determine whether function of the sarcoplasmic reticulum is reduced in obesity.

Using the present experimental results in a computer simulation, we showed that under resting conditions, an isolated reduction in ventricular compliance resulted in near-normal cardiac output. However, cardiac output is maintained at the expense of increased extracellular fluid volume and increased atrial filling pressure. Increased volume together with reduced compliance can result in an abnormally increased filling pressure during maneuvers that increase central blood volume such as passive supine leg raising and supine exercise.3 Furthermore, our simulation also demonstrated that increased diastolic stiffness alone can cause reduced cardiac output and systemic flow during exercise despite increased filling pressure. This suggests that increases in extracellular fluid volume and filling pressure can maintain heart function at rest but that they are insufficient to maintain
adequate pumping capacity during periods of increased demand. In this respect, the computer simulations are consistent with experimental studies. In obese dogs, we have previously shown abnormal cardiovascular responses to exercise that were consistent with a defect in diastolic function. In these animals obesity was associated with increased resting left atrial pressure, an abnormal increase in left atrial pressure during exercise, and frank exercise intolerance. Our computer simulation studies suggest that an isolated reduction in diastolic compliance can be an important component in this reduction of cardiac reserve in obesity.

In the present study, we used the Langendorff isolated heart preparation to assess passive diastolic compliance. This method has been criticized for its lack of reflex and humoral background and feedback. However, this feature is also one of the great strengths of the method in that it allows study of intrinsic heart function without interference from neurohumoral sources. Our analyses, we used both nonnormalized (Figure 1A) and normalized (Figure 1B) pressure-volume data to assess chamber stiffness. Although normalization of data may produce misleading information, both analyses suggested the same conclusion regarding early development of diastolic dysfunction in obesity. Nevertheless, determination of the stress-strain relationship and a myocardial stiffness constant would add another dimension to the characterization of left ventricular function and allow further inferences regarding myocardial functional and structural defects.

In summary, we used the rabbit model of obesity to identify reduced diastolic compliance as a potential factor in abnormalities in diastolic filling seen in obesity. Using the isolated heart technique, we demonstrated that the passive end-diastolic pressure-volume relationship was shifted to the left as a result of an increase in the modulus of stiffness. An associated computer simulation suggests that a change in compliance alone is sufficient to increase cardiac filling pressure and contribute to the occurrence of congestive heart failure. Taken together, these results suggest that reduced diastolic compliance occurs early in obesity and may be a major factor in the increased risk of congestive heart failure associated with obesity.

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