Dietary-Induced Obesity Hastens the Progression From Concentric Cardiac Hypertrophy to Pump Dysfunction in Spontaneously Hypertensive Rats.

Olebogeng HI Majane, Leanda Vengethasamy, Eugene F du Toit*, Siyanda Makaula, Angela J Woodiwiss, Gavin R. Norton.

From the Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, University of the Witwatersrand, Johannesburg, and the Department of Biomedical Sciences*, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa.

Abbreviated title: obesity and pump dysfunction

Correspondence and reprint requests: Angela J Woodiwiss and Gavin R Norton. Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, University of the Witwatersrand Medical School, 7 York Road, Parktown, 2193, Johannesburg, South Africa. Tel: +27 11 717 2363/2354, Fax +27 11 717 2153, e-mail: angela.woodiwiss@wits.ac.za and gavin.norton@wits.ac.za

Abstract

We explored whether dietary-induced obesity hastens the transition from concentric left ventricular hypertrophy (LVH) to pump dysfunction in spontaneously hypertensive rats (SHR) and the mechanisms thereof. After feeding rats a diet for 4-5 months, obesity was induced in SHR and Wistar Kyoto (WKY) control rats. Obesity was not associated with abnormal blood glucose control (HbA1c) or with increases in systolic blood pressure (BP). However, in SHR, but not in WKY rats, obesity was associated with a reduced LV chamber systolic function as determined by echocardiography and in isolated perfused heart studies. A marked increase in LV end diastolic diameter and a right shift in the LV diastolic pressure-volume relation were noted in obese SHR, but not in obese WKY rats. Moreover, LV intrinsic myocardial systolic function, as determined from the slope of the linearized LV systolic stress-strain relationship (En), was markedly reduced in obese as compared to lean SHR, whilst En was maintained in obese WKY rats. Obesity increased LV weight, cardiomyocyte width, cardiomyocyte apoptosis (TUNEL), the activity of myocardial matrix metalloproteinases (zymography) and serum leptin concentrations in SHR, but not in WKY rats. In conclusion, SHR are susceptible to the adverse effects of dietary-induced obesity on the heart, an effect that hastens the progression from concentric LVH to pump dysfunction independent of BP changes or alterations in HbA1c. This effect may be mediated through a proclivity of SHR to developing both obesity-induced effects on cardiomyocyte apoptosis and activation of myocardial collagenases through leptin resistance and obesity-induced hypertrophy.

Key words: obesity, pump dysfunction, spontaneously hypertensive rats, dilatation.

Introduction

Obesity is an independent risk factor for heart failure.[1-3] As adjustments for cardiac systolic chamber function abolish the relationship between obesity and heart failure,[2] it is possible that heart failure in obesity is caused by pump dysfunction rather than diastolic abnormalities. There is increasing evidence from both human[4,5] and animal studies[6,7] that obesity is associated with myocardial contractile disturbances. However, a cause-effect relationship between obesity and cardiac systolic dysfunction is controversial. Indeed, weight loss produced by lifestyle modification or gastric bypass does not influence left ventricular (LV) myocardial systolic dysfunction.[8,9] Moreover, although some studies have reported a decreased LV pump function associated with obesity,[10-13] the majority indicate a normal or even increased LV pump function.[4,5,14-22] As pump dysfunction is an established predictor of the development of heart failure,[23] clarity on the role of obesity in the development of pump dysfunction is required.

Obesity may augment the impact of hypertension on LV hypertrophy (LVH),[24,25] and an increased LV mass is a risk factor for the development of pump dysfunction.[26] We therefore hypothesized that obesity may exaggerate the detrimental effects of hypertension on the heart and consequently hasten the transition from concentric LVH to LV dilatation and pump dysfunction. To test this hypothesis we assessed whether spontaneously hypertensive rats (SHR) are susceptible to the potential adverse effects of dietary-induced obesity on the heart and consequently whether dietary-induced obesity in SHR promotes the transition from concentric LVH to LV dilatation and pump dysfunction prior to an age when SHR normally develop systolic decompensation.[27] We also assessed the mechanisms thereof.

Methods

This study was conducted in accordance with the Principles of Laboratory Animal Care of the National Society for Medical Research and the Guide for the Care and use of

Laboratory Animals of the National Academy of Sciences (NIH publication no 80-23, revised 1985) and was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (Approval number: 2006/99/3). Eight month old SHR and age-matched WKY rats were assigned to receive an obesity-inducing or control diet for 5 months.[22] The dietary intervention was initiated at an age when SHR have concentric LVH without pump dysfunction or increases in diastolic chamber stiffness.[27,28] The experimental diet resembles a Western-type diet,[29] the constituents of which are described in an on-line supplement. The diet is designed to induce hyperphagia,[29] resulting in a greater energy intake (570±23 kJ/day) as compared to the control group (371±18 kJ/day), with similar increments in caloric intake noted in both SHR and WKY rats. Differences in micronutrient (vitamins and minerals) intake produced by dilution of the diet by addition of carbohydrates and fats, does not modify either body size or cardiac function.[22]

The impact of the diet on adipose tissue was assessed from weekly body weights and visceral fat (retroperitoneal and omental) weight determined at the end of the study. Tail cuff systolic blood pressure (BP) was determined at regular intervals during the study using a previously described technique.[30] Fasting blood samples were obtained at the time of thoracotomy when the heart was removed for perfusion studies. Blood glucose control was determined from percentage glycosylated haemoglobin (HbA1c).[31] Serum insulin and leptin concentrations were measured using ultrasensitive rat insulin (DRG Instruments GmbH, Germany) and rat leptin (Assay designs, Ann Arbor, USA) enzyme immunoassays. Serum non-esterified free fatty acid concentrations were measured using an enzymatic colorimetric assay (Roche, Germany).

Echocardiography. Left ventricular systolic function and chamber dimensions were determined *in vivo* as previously described[32] in anaesthetized rats (50 mg/kg of ketamine and 3 mg/kg of xylazine) using two-dimensional targeted M-mode

echocardiography (7.5 MHz transducer and a Hewlett Packard Sonos 2500 sector scanner) according to the American Society of Echocardiography convention (see on-line supplement for details).

Isolated, perfused heart preparations. As LV function assessed using echocardiography is load and heart rate-dependent, we also assessed LV function and remodeling *ex vivo* as previously described,[32,33] (see on-line supplement for details) using load-independent measures in paced, isolated perfused heart preparations.

Cardiomyocyte necrosis and apoptosis. From a longitudinal slice of the LV obtained from the apex to the base through the LV free wall, a pathological score was determined using a previously described approach (see on-line supplement).[27] The degree of apoptosis was quantified as previously described[34] on 5 μm thick myocardial tissue sections obtained from the same tissue blocks used to assess the pathological score using a TUNEL technique (DeadEnd™ Colorimetric TUNEL system, Promega, Madison, WI, USA) (see on-line supplement for details).

Matrix metalloproteinase activity. To avoid a potential impact of prolonged anesthesia and perfusion of the myocardium with artificial solutions on matrix metalloproteinase (MMP) activity, gelatin zymography was performed as previously described[35] on tissue from the lateral wall of the LV collected from rats that had not undergone hemodynamic assessment (see on-line supplement for details).

Myocardial collagen. Samples of LV tissue were weighed and stored at -70°C prior to tissue analysis. Myocardial hydroxyproline concentration ([HPRO]) was determined after acid (HCl) hydrolysis.[30-34] Myocardial collagen was also extracted and digested with cyanogen bromide (CNBr) and subjected to acid hydrolysis and [HPRO] determination.[30,33,34] The amounts of non-cross-linked (soluble) and cross-linked (insoluble) collagen in the myocardium were ascertained based on the solubility of myocardial collagen to CNBr digestion.[30,33,34]

Myocyte isolation and assessment of cell morphometry. Cardiomyocyte morphology was determined on isolated cardiomyocytes using image analysis. Cell isolation was performed as previously described[36,37] (see on-line supplement) and the technique for image analysis is outlined in an on-line supplement.

Data analysis. Regression analysis was used to determine the lines of best fit for the cardiac function relations. All data are presented as mean±SEM. Comparisons between groups were made with a two way ANOVA.

Results

Characteristics of the obesity model. At the initiation of the study, WKY rats were heavier than SHR, but otherwise body weights were similar between groups of animals assigned to dietary groups (data not shown). The diet produced a modest increase in body weight in both SHR and WKY groups, but almost doubled visceral fat content (Table 1). At the initiation of the study, systolic BP values were higher in SHR as compared to WKY rats, but were otherwise similar between groups of animals assigned to dietary groups (data not shown). The experimental diet failed to modify systolic BP in either group (Table 1). Furthermore, blood glucose control, as indexed by HbA1c measurements, was unchanged in either SHR or WKY rats receiving the experimental diet (Table 1). Consistent with a reduced body size and hence adipose tissue content in SHR, serum leptin concentrations were reduced in SHR (Table 1). Although neither serum free fatty acid nor insulin concentrations were altered by the presence of obesity, the experimental diet increased plasma leptin concentrations in SHR, but not in WKY rats (Table 1).

Cardiac weight and cardiomyocyte size. Despite SHR having increased systolic BP values, as SHR were considerably smaller than WKY rats, SHR receiving the control diet had similar absolute heart and LV weights and cardiomyocyte length and width values as compared to WKY rats (Table 1). However, when normalised for body weight

differences, SHR had marked increases in LV weights as compared to WKY rats (Table 1). The experimental diet increased LV weight and cardiomyocyte width, but not length in SHR (Table 1), with the effect on LV weight achieving statistical significance when LV weights were normalized for growth effects as determined from tibial length measurements ($p<0.05$ for the interaction between the presence of hypertension and the diet) (Table 1). However, the diet failed to increase heart and LV weights or cardiomyocyte width and length in WKY rats (Table 1). No differences in LV wet-to-dry weight ratios were noted between the groups (WKY Control=4.97±0.08, WKY diet=4.84±0.09, SHR Control=5.00±0.07, SHR diet=5.08±0.04).

Left ventricular pump function. The SHR receiving the control diet had a greater FSend (Figure 1, right lower panel) and a left shift in the LV systolic pressure-volume relationship (Figure 1, upper panel) as compared to WKY rats receiving the control diet. However, pump function, as assessed using the load and heart rate-independent index of function, LV Ees (Figure 1, left lower panel), was unchanged in SHR as compared to WKY rats receiving a control diet. The experimental diet given to SHR resulted in a decrease in LV systolic chamber function as indicated by a decline in both FSend, and a right shift in the LV systolic pressure-volume relationship, a change attributed to a decrease in the slope of this relationship (LV Ees) (Figure 1). In contrast, neither FSend nor the LV systolic pressure-volume relationship or its slope (LV Ees) was altered by feeding the experimental diet to WKY rats (Figure 1).

Left ventricular instrinsic myocardial systolic function. The SHR and WKY rats receiving the control diet had a similar intrinsic myocardial systolic function (systolic stress-strain relationships and LV En were comparable) (Figure 2). The experimental diet given to SHR produced a striking decrease in intrinsic myocardial systolic function as indicated by a right shift in the LV systolic stress-strain relation, an effect that was attributed to a decrease in the slope of this relationship (LV En) (Figure 2). However, the

experimental diet failed to modify intrinsic myocardial systolic function in WKY rats (Figure 2).

Left ventricular chamber remodeling. Although SHR had a left shift in the LV diastolic pressure-volume relationship (Figure 3, left upper panel), and a decrease in LV V_0 (Figure 3, left lower panel) and LV EDD (Table 1), after adjustments of LV volumes for body weight, SHR receiving the control diet had similar LV diastolic pressure-volume relations and LV V_0 values as WKY rats (Figure 3, right panels). Despite having similar LV weights as age-matched WKY rats, SHR had increased LV end diastolic posterior and relative wall thickness values (Table 1). The experimental diet given to SHR resulted in a right shift in the LV diastolic pressure-volume relationships (Figure 3, upper panels), an increase in LV V_0 (Figure 3, lower panels), an increase in LV EDD (Table 1) and a decrease in LV posterior and relative wall thickness (Table 1). In contrast, the experimental diet given to WKY rats had no effect on the LV diastolic pressure-volume relationships, LV V_0, LV EDD, or LV posterior and relative wall thickness (Figure 3 and Table 1).

Myocardial apoptosis, necrosis and interstitial changes. The SHR receiving a control diet had a similar percentage cardiomyocyte apoptotic nuclei and myocardial MMP-2 activity as WKY rats receiving a control diet (Figure 4). Administration of the experimental diet to SHR however produced an increase in cardiomyocyte apoptosis and myocardial MMP-2 activity as compared to SHR receiving the control diet (Figure 4). In contrast, the experimental diet failed to modify the degree of cardiomyocyte apoptosis or myocardial MMP-2 activity in WKY rats (Figure 4). In contrast to effects on the index of cardiomyocyte apoptosis, the experimental diet failed to influence the myocardial pathological score in either SHR or WKY rats (Table 2).

SHRs receiving a control diet had increased myocardial collagen concentrations, as indexed by HPRO measurements, as compared to WKY rats receiving a control diet,

a change attributed to an increase in myocardial cross-linked collagen concentrations (increased insoluble HPRO concentration) (Table 2). The preferential accumulation of cross-linked myocardial collagen in SHR was attributed to an increase in myocardial collagen cross-linking as detected by a decrease in myocardial collagen soluble to digestion by CNBr (Table 2). As a consequence of a decrease in myocardial collagen solubility in SHR, the non-cross-linked form of HPRO was unchanged in SHR as compared to WKY rats receiving the control diet (Table 2). Administration of the experimental diet to either SHR or WKY rats failed to modify HPRO concentrations or to alter the degree of myocardial collagen cross-linking (Table 2).

Discussion

The main findings of the present study are as follows: Predominantly visceral obesity induced by feeding a diet designed to produce an increased caloric intake, was associated with the premature progression from compensated LVH to LV pump dysfunction in SHR. In contrast, a comparable degree of dietary-induced obesity failed to produce adverse effects on cardiac function in normotensive WKY rats. The dietary-induced change in pump function in SHR occurred in the absence of further peripheral systolic BP changes or changes in blood glucose control (HbA1c). Dietary-induced pump dysfunction in SHR was noted both *in vivo* (endocardial fractional shortening) and *ex vivo* using a load-independent assessment of chamber function (end systolic chamber elastance). Further, dietary-induced pump dysfunction in SHR, but not WKY rats was associated with a reduced intrinsic myocardial contractile function (end systolic myocardial elastance) and marked LV dilatation (increased chamber diameters and a right shift in the LV diastolic pressure-volume relationship), changes that were associated with increases in cardiomyocyte apoptosis and myocardial MMP-2 activation in SHR, but not in WKY rats. However, dietary-induced pump dysfunction in SHR was not associated with excessive myocardial necrosis, changes in myocardial collagen

concentrations, or alterations in the quality of myocardial collagen, although it is possible that at the initiation of the dietary intervention, SHR may have had myocardial collagen changes. The modest degree of generalised obesity produced by the experimental diet failed to translate into changes in serum leptin concentrations or LV weight in WKY rats, but nevertheless resulted in increases in serum leptin concentrations and an exaggerated degree of LVH and an increased cardiomyocyte width in SHR.

Albeit that the results described in the present study were obtained in SHR and hence may not be extrapolated to other models, this is the first prospective study to show that obesity, in the absence of hyperglycemia, can promote marked LV dilatation and pump dysfunction. Clinical studies demonstrating obesity-induced myocardial contractile disturbances have been conducted using case-control designs rather than prospective studies, and have failed to show that these changes translate into clinically relevant alterations in pump function.[4,5] Further, whether the association between obesity and myocardial contractile disturbances in clinical studies reflects cause and effect relations is controversial as weight loss produced either by lifestyle modification or gastric bypass does not influence LV myocardial systolic dysfunction.[8,9] Although prospective studies in euglycemic animals indicate that obesity is associated with myocardial contractile disturbances,[6,7] there have been no reports to indicate that these changes translate into clinically relevant abnormalities in pump function. Although the present study clearly shows that obesity can promote myocardial contractile disturbances that translate into pump dysfunction in SHR even in the absence of hyperglycemia or further increases in BP, the caveat is that these deleterious effects are mediated by an interaction between obesity and hypertension. Importantly, in the absence of hypertension in WKY rats, the same degree of obesity produced no discernable adverse effects on the heart.

As LVH is a risk factor for the development of pump dysfunction,[26] one potential mechanism responsible for the obesity-induced effect on pump dysfunction in SHR noted in the present study is an interaction between obesity and hypertension to promote LVH. Indeed, in agreement with previous clinical studies,[24,25] in the present study an interaction between hypertension and obesity was noted to contribute toward increases in LV weight and cardiomyocyte width in SHR. One potential mechanism for this effect could be through an increased propensity for SHR to develop leptin resistance, a change which promotes LVH.[38] Indeed, in the present study dietary-induced obesity was associated with an increase in serum leptin concentrations in SHR, but not in WKY rats. This effect in SHR occurred despite similar increases in body weight and visceral fat content in the SHR and WKY groups. The increased serum leptin concentrations in SHR could be explained by the presence of leptin resistance.

Although obesity-induced pump dysfunction in SHR in the present study cannot be attributed to further increments in BP as measured in the periphery by tail-cuff techniques, we cannot exclude the possibility that increases in central BP may have occurred. Indeed, excess adiposity is an independent predictor of increases in arterial stiffness[39] and changes in arterial stiffness which influence central BP through early reflective waves are not closely emulated by peripheral BP values.[40]

In agreement with previous studies conducted in euglycemic models of obesity in rodents,[41,42] in the present study obesity was associated with increases in cardiomyocyte apoptosis, but only in SHR. In this regard, the excessive cardiomyocyte apoptosis in the SHR, but not the WKY rats, closely tracked intrinsic myocardial and pump dysfunction. Hence, dietary-induced pump dysfunction may be attributed in-part to excess cardiomyocyte apoptosis, a change that is now considered to be an important pathophysiological process in the development of heart failure. As LVH is associated with cardiomyocyte apoptosis,[43] the excessive apoptotic changes noted in obese SHR,

but not in WKY rats in the present study may be attributed to an augmented LVH in SHR. Alternatively, excessive cardiomyocyte apoptosis in obese SHR may relate to the potential leptin resistance noted in these rats and the associated changes in myocardial substrate metabolism that may occur with leptin resistance,[41] an effect that may result in ectopic lipid overload in cardiomyocytes (lipoapoptosis).[42] Nevetherless, other mechanisms may also play a role, including increases in circulating aldosterone concentrations noted to occur in obese SHR.[44]

Importantly, in the present study the degree of dietary-induced LV dilatation in SHR exceeded that which could be explained by increases in body size, as marked right shifts in LV diastolic pressure-volume relations were noted in SHR fed the experimental diet even after normalising LV volumes to 100 g body weight. As previously demonstrated[32] cardiac dilatation is likely to contribute toward progressive pump dysfunction and heart failure in pressure overload states. Whether obesity-induced cardiac dilatation noted in SHR in the present study is an indirect consequence of decreases in intrinsic myocardial contractile function or through direct effects of the diet could not be determined. However, the cellular change responsible for cardiac dilatation in the present study, appeared to be through the actions of activated collagenases (MMP-2)[45] as opposed to changes in myocardial collagen concentrations,[27] or reductions in myocardial collagen cross-linking.[27,33,34]

The limitations of the present study include the following. First, as the present study was conducted in a genetic model of hypertension, the differences noted between the effects of dietary-induced obesity on the myocardium in SHR and WKY rats, may be attributed to molecular or cellular differences rather than to blood pressure effects *per se.* In this regard, further studies conducted in non genetic forms of pressure overload hypertrophy are required to distinguish between these effects. Second, although the experimental diet had only a 6% higher fat content (weight/weight) as compared to the

control diet, we were unable to evaluate whether the fatty acid content of the experimental diet as opposed to the presence of obesity *per se* could explain the adverse effects on cardiac function noted in SHR in the present study. In this regard, a high fat diet (20% weight/weight) rich in linoleic acid may be beneficial, whilst a lard diet (20% weight/weight) may be detrimental to cardiac function in SHR.[46] Last, we did not study a food-restricted, weight-matched group of rats receiving the experimental diet, as we had no method of controlling for the effect of food restriction *per se*. Thus, the effects of the experimental diet in the present study may have been as a consequence of the constituents of the diet as opposed to the obesity that the diet produced. Nevertheless, a previous study has shown that the adverse effects of dietary-induced obesity on the myocardium are likely to be attributed to the obesity rather than to the dietary constituents.[47]

Perspectives

The present study indicates that in the absence of further increases in peripheral BP or the presence of hyperglycemia, SHR are susceptible to the adverse effects of an obesity-inducing diet, which promotes the premature progression from concentric LVH to pump dysfunction in this animal model of genetic hypertension. This effect occurs through both decreases in intrinsic myocardial systolic function and through cardiac dilatation, changes associated with cardiomyocyte apoptosis and myocardial MMP-2 activation, but not through cardiomyocyte necrosis, or alterations in the quantitative or qualitative characteristics of the myocardial interstitium. The potential mechanisms of these effects may include a propensity for SHR to develop leptin resistance and a proclivity for obesity to exaggerate hypertensive LVH in SHR.

Acknowledgements

This work was supported by grants from the South African National Research Foundation awarded to OHIM and AJW. LV was supported by a South African Medical Research Council studentship.

Sources of Funding

This work was supported by grants from the South African National Research Foundation awarded to OHIM and AJW. LV was supported by a South African Medical Research Council studentship.

References

1. Kenchaiah S, Evans JC, Levy D, Wilson PWF, Benjamin EJ, Larson SD, Kannel WB, Vasan RS. Obesity and the risk of heart failure. *New Engl J Med*. 2002;347:305-313.
2. Bahrami J, Bluemke DA, Kronwal R, Bertoni AG, Lloyd-Jones DM, Shahar E, Szklo M, Lima JAC. Novel metabolic risk factors for incident heart failure and their relationship with obesity. *J Am Coll Cardiol*. 2008;51:1775-1783.
3. Ingelsson E, Sundstrom J, Arnlov J, Zethelius B, Lind L. Insulin resistance and risk of congestive heart failure. *J Am Med Ass.* 2005;294:334-341.
4. Peterson LR, Waggoner AD, Schechtmann KB, Meyer T, Gropler RJ, Barzilia B, Davila-Romain VG. Alterations in left ventricular structure and function in young healthy obese women. *J Am Coll Cardiol.* 2004;43:1399-1404.
5. Wong CY, O'Moore-Sullivan T, Leano R, Byrne N, Beller E, Marwick TH. Alterations of left ventricular myocardial characteristics associated with obesity. *Circulation.* 2004;110:3081-3087.
6. Dong F, Zhang X, Yang X, Esberg LB, Yang H, Zhang Z, Culver B, Ren J. Impaired cardiac contractile function in ventricular myocytes from leptin deficient ob/ob obese mice. *J Endocrinology.* 2006;188:25-36.
7. Ren J, Sowers JR, Walsh MF, Brown RA. Reduced contractile response to insulin and IGF-I in ventricular myocytes from genetically obese Zucker rats. *Am J Physiol*. 2000;279:H1708-H1714.
8. Skilton MR, Sieveking DP, Harmer JA, Franklin J, Loughnan G, Nakhla S, Sullivan DR, Caterson JD, Celermajer DS. The effects of obesity and non-pharmacological weight loss on vascular and ventricular function and structure. *Diabet Obes Metab*. 2007;10:874-884.

9. Wong CY, Byrne NM, O'Moore-Sullivan T, Hills AP, Prins JB, Marwick TH. Effect of weight loss due to lifestyle intervention on subclinical cardiovascular dysfunction in obesity (body mass index >30 kg/m^2). *Am J Cardiol*. 2006;98:1593-1598.

10. Scaglione R, Dichiara MA, Indovina A, Lipari R, Ganguzza A, Parrinello G, Capuana G, Merlino G, Licata G. Left ventricular diastolic and systolic function in normotensive obese subjects: influence of degree and duration of obesity. *Eur Heart J*. 1992;13:738-742.

11. Karason K, Wallentin I, Larsson B, Sjostrom L. Effects of obesity and weight loss on cardiac function and valvular performance. *Obes Res.* 1998;6:422-429.

12. Alpert MA, Lambert CR, Panayiotou H, Terry BE, Cohen MV, Massey CV, Hashimi MW, Mukerji V. Relation of duration of morbid obesity to left ventricular mass, systolic function, and diastolic filling, and effect of weight loss. *Am J Cardiol*. 1995;76:1194-1197.

13. Alpert MA, Terry BE, Lambert CR, Kelly DL, Panayiotou H, Mukerji V, Massey CV, Cohen MV. Factors influencing left ventricular systolic function in non hypertensive morbidly obese patients and effect or weight loss. *Am J Cardiol*. 1993;15:733-737.

14. Pascual M, Pascual DA, Soria F, Vicente T, Hernandez AM, Tebar FJ, Valdes M. Effects of isolated obesity on systolic and diastolic left ventricular function. *Heart.* 2003;89:1152-1156.

15. Devitiis O, Fazio S, Petitto M, Maddalena G, Contaldo F, Mancini M. Obesity and cardiac function. *Circulation.* 1981;64:477-482.

16. Zarich SW, Kowalchuk GJ, McGuire MP, Benotti PN, Mascioli EA, Nesto RW. Left ventricular filling abnormalities in asymptomatic morbid obesity. *Am J Cardiol*. 1991;68:377-381.

17. Stoddard MF, Tseuda K, Thomas M, Dillon S, Kupersmith J. The influence of obesity on left ventricular filling and systolic function. *Am Heart J*. 1992;124:694-699.

18. De Simone G, Deveroux RB, Mureddu GF, Roman MJ, Ganau A, Alderman MH, Contaldo F, Laragh JH. Influence of obesity on left ventricular midwall mechanics in arterial hypertension. *Hypertension*. 1996;28:276-283.

19. Mureddu GF, de Simone G, Greco R, Rosato GF, Contaldo F. Left ventricular filling pattern in uncomplicated obesity. *Am J Cardiol*. 1996;77:509-514.

20. Iacobellis G, Ribaudo MC, Leto G, Zappaterreno A, Vecci E, DiMario U, Leonetti F. Influence of excess fat on cardiac morphology and function. Study in uncomplicated obesity. *Obes Res*. 2002;10:767-773.

21. Carroll JF, Zenebe WJ, and Strange TB. Cardiovascular function in a rat model of diet induced obesity. *Hypertension*. 2006;48:65-72.

22. du Toit EF, Smith W, Muller C, Strijdom H, Stouthammer B, Woodiwiss AJ, Norton GR, Lochner A. Myocardial susceptibility to ischemic-reperfusion injury in a pre-diabetic model of dietary-induced obesity. *Am J Physiol*. 2008;294:H2336-H2343.

23. Hunt SA. American College of Cardiology;American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). *J Am Coll Cardiol*.2005;46:e1– 82.

24. Avelar E, Cloward TV, Walker JM, Farney RJ, Strong M, Pendleton RC, Segerson N, Adams TD, Gress RE, Hunt SC, Litwin SE. Left ventricular hypertrophy in severe obesity. Interactions among blood pressure, nocturnal hypoxemia, and body mass. *Hypertension*. 2007;49:34-39.

25. Norton GR, Majane OHI, Libhaber E, Libhaber C, Maseko MJ, Makaula S, Libhaber C, Woodiwiss AJ. The relationship between blood pressure and left ventricular mass index is dependent on an excess adiposity*. J Hypertens.* 2009;27:1873-1883.

26. Drazner MH, Rame JE, Marino EK, Gottdiener JS, Kitzman DW, Gardin JM, Manolio TA, Dries DL, Siscovick DS. Increased left ventricular mass is a risk factor for the development of a depressed left ventricular ejection fraction within five years: the Cardiovascular Health Study. *J Am Coll Cardiol*. 2004;43:2207-2215.

27. Tsotetsi OJ, Woodiwiss AJ, Netjhardt M, Qubu D, Brooksbank R, Norton GR. Attenuation of cardiac failure, dilatation, damage, and detrimental interstitial remodeling without regression of hypertrophy in hypertensive rats. *Hypertension*. 2001;38:846–851.

28. Norton GR, Veliotes DGA, Osadchii O, Woodiwiss AJ, Thomas DP. Susceptibility to systolic dysfunction in the myocardium from chronically infracted spontaneously hypertensive rats. *Am J Physiol Heart and Circ Physiol*. 2008;294:H372-H378.

29. Pickavance L, Tadayyon M, Widdowson PS, Buckingham RE, Wilding JP. Therapeutic index for rosiglitazone in dietary obese rats: separation of efficacy and haemodilution. *Br J Pharmacol*. 1999;128:1570-1576.

30. Norton GR, Tsotetsi J, Trifunovic B, Hartford C, Candy GP, Woodiwiss AJ. Myocardial stiffness is attributed to alterations in cross-linked collagen rather

than total collagen or phenotypes in spontaneously hypertensive rats. *Circulation*. 1997;96:1991-1998.

31. Norton GR, Candy GP, Woodiwiss AJ. Aminoguanidine prevents the decreased myocardial compliance produced by steptozotocin-induced diabetes mellitus in rats. *Circulation*. 1996;93:1905-1912.

32. Norton GR, Woodiwiss AJ, Gaasch WH, Mela T, Chung ES, Aurigemma GP, Meyer TE. Heart failure in pressure overload hypertrophy. The relative roles of ventricular remodeling and myocardial dysfunction. *J Am Coll Cardiol*. 2002;39:664-671.

33. Woodiwiss AJ, Tsotetsi OJ, Sprott S, Lancaster EJ, Mela T, Chung ES, Meyer TE, Norton GR. Reduction in myocardial collagen cross-linking parallels left ventricular dilatation in rat models of systolic chamber dysfunction. *Circulation*. 2001;103:155-160.

34. Veliotes DGA, Woodiwiss AJ, Deftereos DAJ, Gray D, Osadchii O, Norton GR. Aldosterone receptor blockade prevents the transition to cardiac pump dysfunction induced by ß-adrenoreceptor activation. *Hypertension*. 2005;45:914-920.

35. Tyagi SC, Matsubara L, Weber KT. Direct extraction and estimation of collagenase(s) activity by zymography in microquantities of rat myocardium and uterus. *Clin Biochem*. 1993;26:191-198.

36. Woodiwiss AJ, Honeyman TW, Fenton RA, Dobson JG. Adenosine A2a receptor activation enhances cardiomyocyte shortening via Ca2+-independent and –dependent mechanisms. *Am J Physiol.* 1999;276:H1434-H1441.

37. Diffee GM, Nagle GF. Exercise training alters length dependence of contractile properties in rat myocardium. *J Appl Physiol.* 94:1137-1144, 2003.

38. Barouch LA, Berkowitz DE, Harrison RW, O'Donnell CP, Hare JM. Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice. *Circulation.* 2003;108:754-759.

39. Majane OHI, Woodiwiss AJ, Maseko MJ, Crowther N, Dessein P, Norton GR. Impact of age on the independent association of adiposity with pulse wave velocity in a population sample of African ancestry. *Am J Hypertens*. 2008;21:936-942.

40. Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur Heart J*. 1993;14:160-167.

41. Barouch LA, Gao D, Miller KL, Xu W, Phan AC, Kittleson MM, Minhas KM, Berkowitz DE, Wei C, Hare JM. Cardiac myocyte apoptosis is associated with increased DNA damage and decreased survival in murine models of obesity. *Circ Res*. 2006;98:119-124.

42. Zhou Y-T, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: Implications for human obesity. *Proc Nat Acad Sci*. 2000;97:1784-1789.

43. Wang Y, Huang S, Sah VP. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein-kinase family. *J Biol Chem*. 1998;273:2161-2168.

44. Nagase M, Yoshida S, Nagase T, Gotoda T, Ando K, Fijita T. Enhanced aldosterone signaling in the early nephropathy of rats with metabolic syndrome: Possible contribution of fat derived factors. *J Am Soc Nephrol*. 2006;17:3438-3446.

45. Spinale FG. Matrix metalloproteinases: regulation and dysregulation in the failing heart. *Circ Res.* 2002; 90: 520-530.

46. Chicco AJ, Sparagna GC, McCune SA, Johnson CA, Murphy RC, Bolden DA, Rees ML, Gardner RT, Moore RL. Linoleate-rich high-fat diet decreases mortality in hypertensive heart failure rats compared with lard and low-fat diets. *Hypertension.* 2008;52:549-555.

47. Fang CX, Dong F, Thomas DP, Ma H, He L, Ren J. Hypertrophic cardiomyopathy in high-fat diet-induced obesity: role of suppression of forkhead transcription factor and atrophy gene transcription. *Am J Physiol*. 2008;295:H1206-H1215.

Figure 1. Effect of an obesity-inducing diet (Diet) on left ventricular (LV) pump function in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats as determined *ex vivo* from LV systolic end pressure-volume relations (upper panel) and the slope of these relations (LV end systolic elastance- LV Ees) (lower left panel) and *in vivo* from LV endocardial fractional shortening (FSend) measurements (lower right panel). * $p<0.05$ for an interaction between the presence of hypertension (SHR vs WKY) and diet category.

Figure 2. Effect of an obesity-inducing diet (Diet) on left ventricular (LV) intrinsic myocardial systolic function in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats as determined *ex vivo* from LV systolic stress-strain relations (upper panel) and the slope of these relations (LV myocardial end systolic elastance- LV En) (lower panel). * $p<0.005$ for an interaction between the presence of hypertension (SHR vs WKY) and diet category.

Figure 3. Effect of an obesity-inducing diet (Diet) on left ventricular (LV) end diastolic pressure-volume relations (upper panels), and the volume intercept (LV Vo) of these relations (lower panels), before (left panels) and after (right panels) normalising LV volumes to 100 g body weight in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats. Left lower panel: * $p<0.005$, SHR control vs other groups. Right lower panel: * $p<0.005$ for an interaction between the presence of hypertension (SHR vs WKY) and diet category.

Figure 4. Effect of an obesity-inducing diet (Diet) on left ventricular cardiomyocyte apoptosis (TUNEL) (upper panel) and myocardial matrix metalloproteinase 2 activity (lower panel) in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats.

* $p<0.005$ for an interaction between the presence of hypertension (SHR vs WKY) and diet category.

Table 1. Effect of an obesity-inducing diet (Diet) on morphological, blood and hemodynamic characteristics in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats.

	WKY		SHR	
Characteristic	Control (n=10)	Diet (n=9)	Control (n=10)	Diet (n= 10)
Final body weight (BW)(g)	484±9	527±8**	362±5†	400±6**†
Tibial length (cm)	4.61±0.12	4.39±0.12	4.68±0.17	4.37±0.12
Body weight/tibial length (/10)	10.6±0.3	12.2±0.5**	7.6±0.3†	8.7±0.2**†
Visceral fat (g)	19.4±1.5	29.4±1.3**	9.6±0.7†	17.2±0.7**†
Systolic BP (mm Hg)	124±4	122±4	184±5†	186±3†
Glycated hemoglobin (%)	4.61±0.22	4.42±0.06	4.78±0.08	4.66±0.09
Heart weight (g)	1.41±0.15	1.52±0.07	1.52±0.07	1.70±0.23‡
LV weight (g)	1.17±0.15	1.23±0.05	1.22±0.08	1.34±0.14‡
LV weight/BW (X100)	2.40±0.003	2.33±0.004	3.41±0.004†	3.55±0.002†
LV weight/tibial length (x10)	2.53±0.10	2.82±0.11	2.63±0.008	3.08±0.15†*
Cardiomyocyte width (μm)	17.3±0.4	16.0±0.4	17.1±0.5	19.0±0.7†*
Cardiomyocyte length (μm)	71.7±1.1	73.0±2.1	76.9±2.1	73.9±2.8
LV EDD (mm)	7.51±0.16	7.68±0.27	5.54±0.27§	6.64±0.27
LV ESD (mm)	4.40±0.24	3.85±0.20	1.66±0.19§	2.67±0.40‡
LV posterior wall thick. (mm)	2.00±0.10	2.16±0.13	2.98±0.20§	2.56±0.10
LV relative wall thick. (mm)	0.54±0.04	0.57±0.05	1.04±0.11§	0.79±0.06
Serum leptin (pg/ml)	1143±128	1040±155	531±85§	1014±81
Serum insulin (μg/l)	0.35±0.03	0.34±0.04	0.38±0.06	0.26±0.03
Serum FFA (μmol/ml)	0.38±0.05	0.41±0.04	0.40±0.05	0.51±0.04

BP, blood pressure; LV, left ventricle, EDD, end diastolic diameter; ESD, end systolic diameter; thick, thickness; FFA, non-esterified free fatty acids. * $p<0.05$ ** $p<0.005$ vs SHR Control diet group or WKY Control diet group, † $p<0.001$ vs respective WKY group, ‡ $p<0.05$ vs WKY Control group, §, $p<0.05$ vs other 3 groups.

Table 2. Effect of an obesity-inducing diet (Diet) on left ventricular (LV) necrosis (pathological score) and interstitial characteristics in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats.

	WKY		SHR	
Parameter	Control (n=10)	Diet (n=9)	Control (n=10)	Diet (n=10)
Pathological score	0.88±0.26	0.50±0.18	0.94±0.18	0.88±0.19
[HPRO] (µg.mg^{-1} dry LV)	3.33±0.21	3.16±0.31	8.04±0.54*	9.22±0.43*
% CNBr solubility	28.6±4.33	35.4±4.8	12.3±1.5*	11.0±1.2*
Soluble HPRO (µg.mg^{-1} dry LV)	0.95±0.12	1.08±0.11	0.96±0.12	0.98±0.09
Insoluble HPRO(µg.mg^{-1} dry LV)	2.37±0.20	2.07±0.28	7.16±0.56*	8.23±0.43*

[HPRO], myocardial hydroxyproline concentration; % CNBr solubility, solubility of myocardial collagen to cyanogen bromide digestion. * $p<0.001$ vs respective WKY groups.

Figure 1

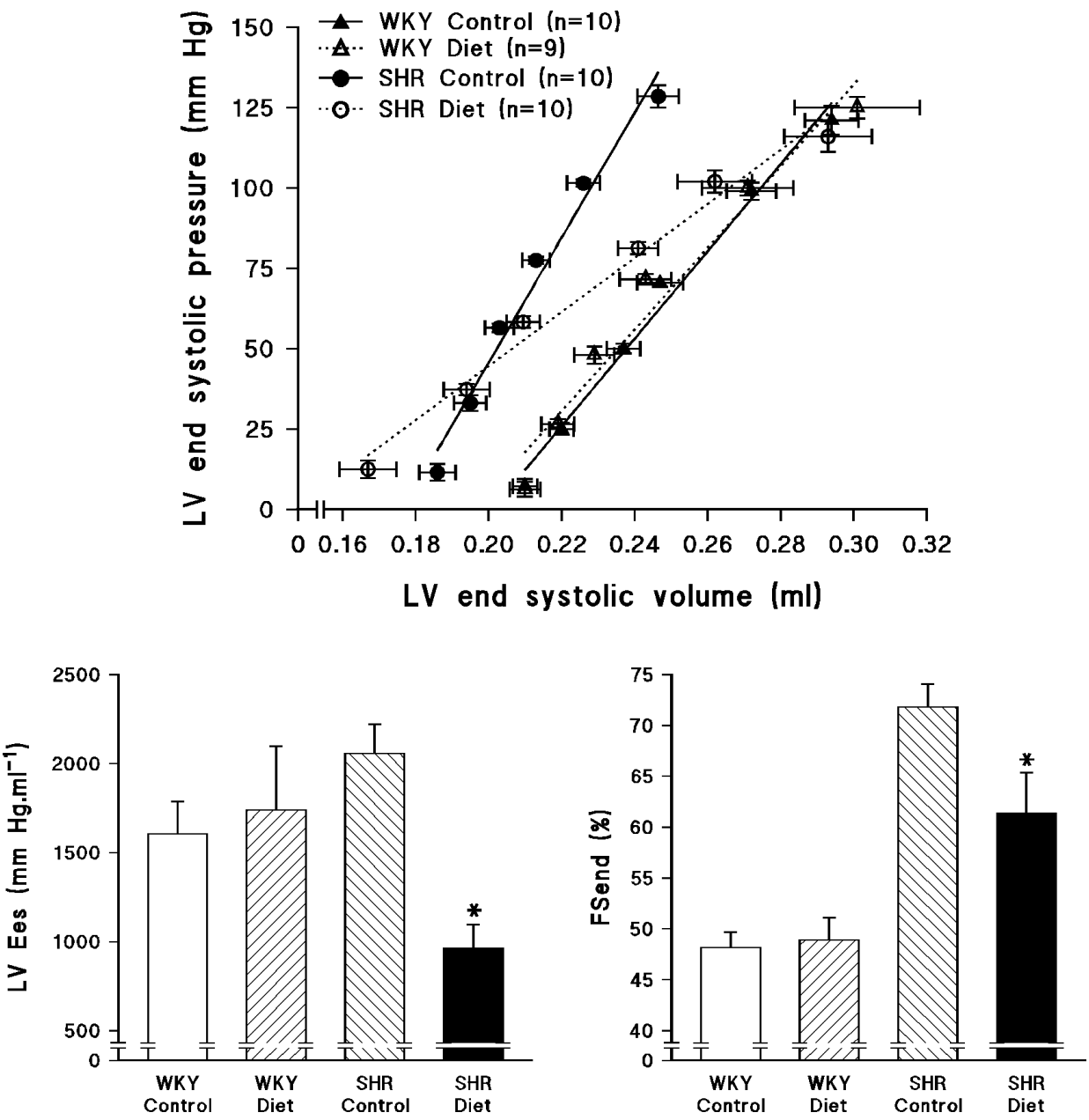
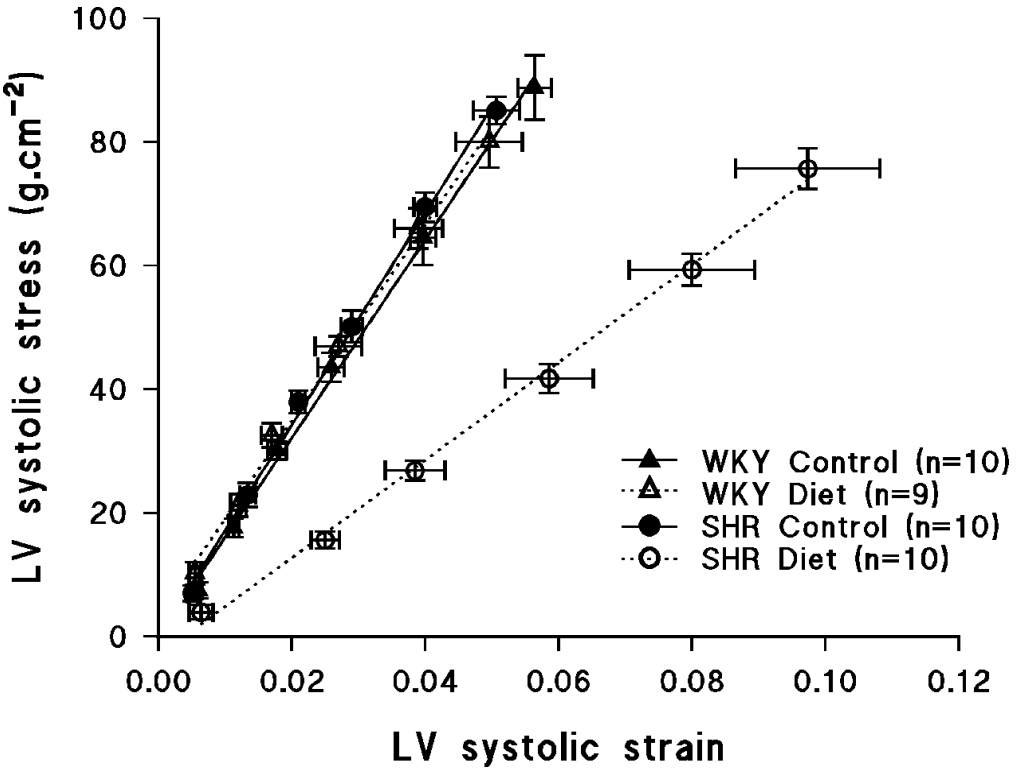

WKY Control (n=10)
WKY Diet (n=9)
SHR Control (n=10)
SHR Diet (n=10)
LV end systolic pressure (mm Hg)
150
125
100
75
50
25
0
0 0.16 0.18 0.20 0.22 0.24 0.26 0.28 0.30 0.32
LV end systolic volume (ml)
LV Ees (mm Hg.ml^{-1})
2500
2000
1500
1000
500
0
*
WKY Control
WKY Diet
SHR Control
SHR Diet
FSend (%)
75
70
65
60
55
50
45
40
0
*
WKY Control
WKY Diet
SHR Control
SHR Diet

Figure 2


LV systolic stress (g.cm^{-2})
100
80
60
40
20
0
0.00
0.02
0.04
0.06
0.08
0.10
0.12
LV systolic strain
WKY Control (n=10)
WKY Diet (n=9)
SHR Control (n=10)
SHR Diet (n=10)

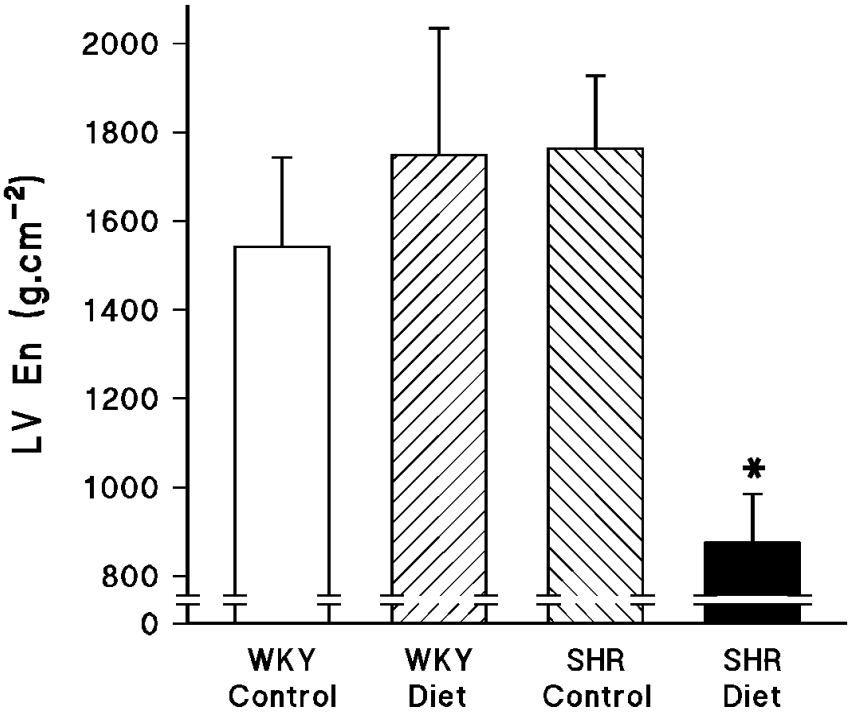
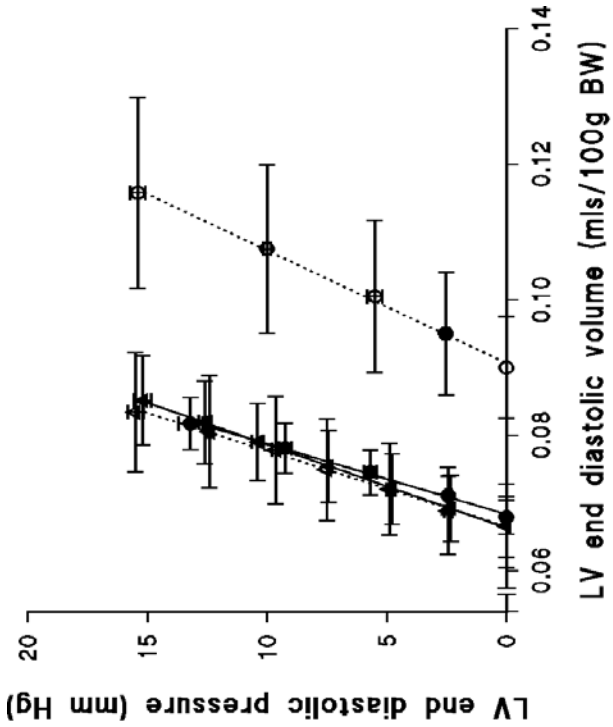
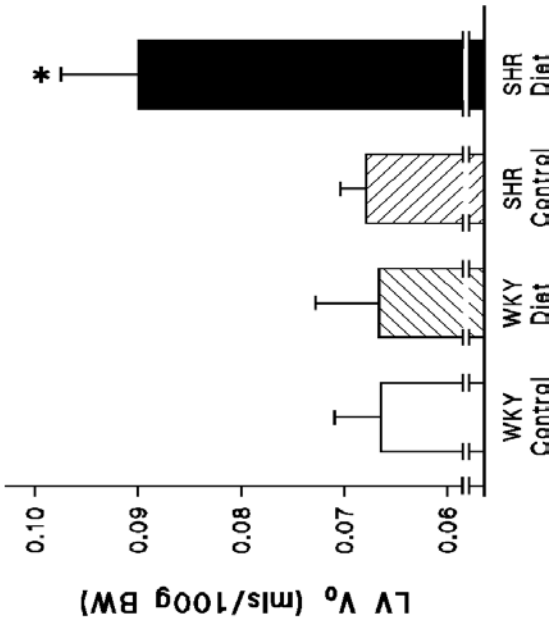
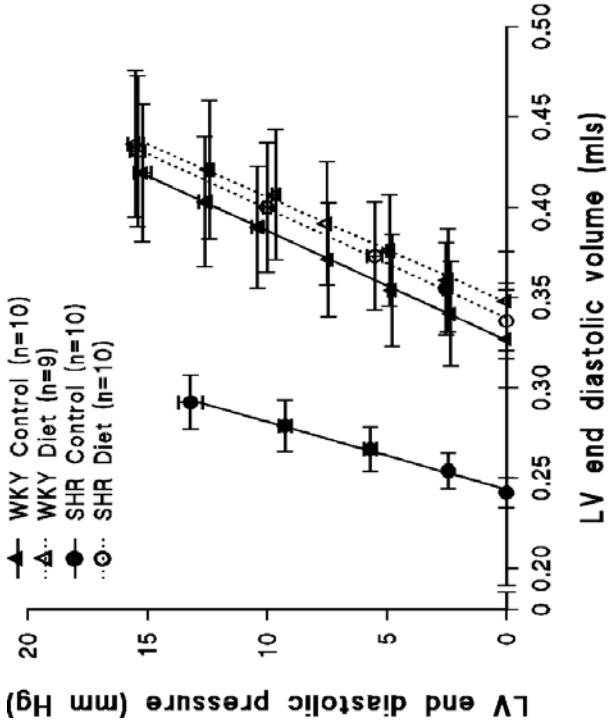

LV En (g.cm^{-2})
2000
1800
1600
1400
1200
1000
800
0
*
WKY Control
WKY Diet
SHR Control
SHR Diet

Figure 3


LV end diastolic pressure (mm Hg)
LV end diastolic volume (mls/100g BW)
0
5
10
15
20
0.06
0.08
0.10
0.12
0.14


LV V0 (mls/100g BW)
0.06
0.07
0.08
0.09
0.10
*
WKY Control
WKY Diet
SHR Control
SHR Diet


WKY Control (n=10)
WKY Diet (n=9)
SHR Control (n=10)
SHR Diet (n=10)
LV end diastolic pressure (mm Hg)
LV end diastolic volume (mls)
0
5
10
15
20
0 0.20
0.25
0.30
0.35
0.40
0.45
0.50

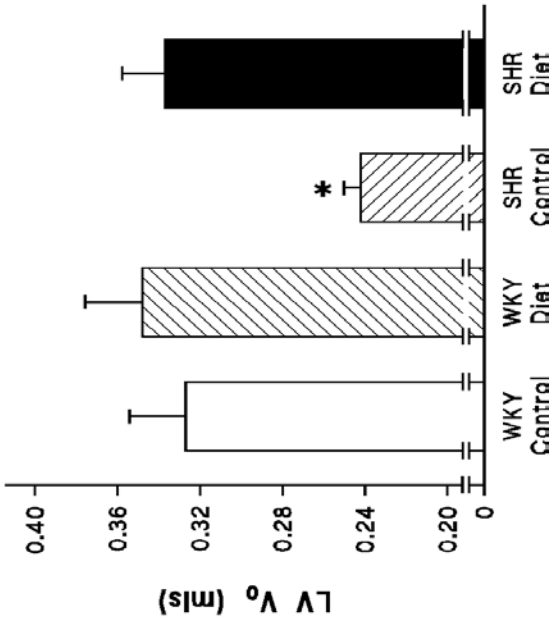
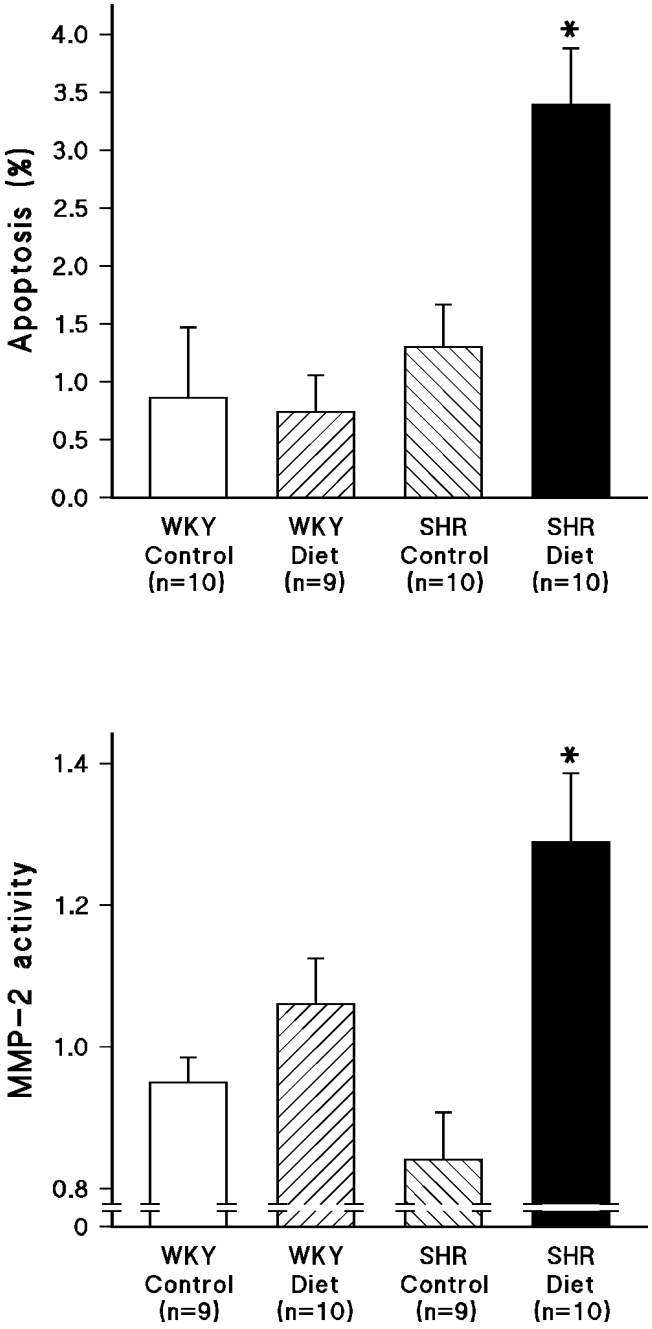

LV V0 (mls)
0
0.20
0.24
0.28
0.32
0.36
0.40
*
WKY Control
WKY Diet
SHR Control
SHR Diet

Figure 4


Apoptosis (%)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
WKY Control (n=10)
WKY Diet (n=9)
SHR Control (n=10)
SHR Diet (n=10)
MMP-2 activity
1.4
1.2
1.0
0.8
0
*
WKY Control (n=9)
WKY Diet (n=10)
SHR Control (n=9)
SHR Diet (n=10)