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

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

Abstract—We explored whether dietary-induced obesity hastens the transition from concentric left ventricular (LV) hypertrophy to pump dysfunction in spontaneously hypertensive rats (SHRs) and the mechanisms thereof. After feeding rats a diet for 4 to 5 months, obesity was induced in SHRs and Wistar-Kyoto (WKY) control rats. Obesity was not associated with abnormal blood glucose control (glycosylated hemoglobin) or with increases in systolic blood pressure. However, in SHRs, 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 SHRs 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 (LV myocardial end systolic elastance), was markedly reduced in obese as compared with lean SHRs, whereas LV myocardial end systolic elastance 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 SHRs but not in WKY rats. In conclusion, SHRs are susceptible to the adverse effects of dietary-induced obesity on the heart, an effect that hastens the progression from concentric LV hypertrophy to pump dysfunction independent of blood pressure changes or alterations in glycosylated hemoglobin. This effect may be mediated through a proclivity of SHRs to developing both obesity-induced effects on cardiomyocyte apoptosis and activation of myocardial collagenases through leptin resistance and obesity-induced hypertrophy. (Hypertension. 2009;54:1376-1383.)

Key Words: obesity ■ ventricular function ■ rats inbred spontaneously hypertensive rats ■ dilatation ■ ventricular remodeling

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 human4,5 and animal studies6,7 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 Because 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 (SHRs) are susceptible to the potential adverse effects of dietary-induced obesity on the heart and, consequently, whether dietary-induced obesity in SHRs promotes the transition from concentric LVH to LV dilatation and pump dysfunction before an age when SHRs normally develop systolic decompensation.27 We also assessed the mechanisms thereof.

Methods

This study was conducted in accordance with the National Society for Medical Research Principles of Laboratory Animal Care and the...
By guest on July 9, 2017

Western-type diet, the constituents of which are described in an Echocardiography convention (please see the online Data Supplement). 2500 sector scanner) according to the American Society of echocardiography (7.5-MHz transducer and a Hewlett Packard S"oetamine and 3 mg/kg of xylazine) using 2D targeted M-mode measured using an enzymatic colorimetric assay (Roche). Serum nonesterified free fatty acid concentrations were measured using ultrasensitive rat insulin (DRG Instruments GmbH) and rat leptin (Assay Designs) enzyme immunoassays. Serum nonesterified free fatty acid concentrations were measured using an enzymatic colorimetric assay (Roche).

Echocardiography

LV systolic function and chamber dimensions were determined in vivo as described previously in anesthetized rats (50 mg/kg of ketamine and 3 mg/kg of xylazine) using 2D targeted M-mode echocardiography (7.5-MHz transducer and a Hewlett Packard Sonos 2500 sector scanner) according to the American Society of Echocardiography convention (please see the online Data Supplement at http://hyper.ahajournals.org for details). The diet is designed to induce hyperphagia, resulting in a greater energy intake (570±23 kJ/d) as compared with the control group (371±18 kJ/d), with similar increments in caloric intake noted in both SHRs and WKY rats. Differences in micronutrient (vitamins and minerals) intake produced by dilution of the diet by addition of carbohydrates and fats do not modify either body size or cardiac function. 

The impact of the diet on adipose tissue was assessed from weekly body weights and visceral fat (retropertioneal 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 technique described previously. Fasting blood samples were obtained at the time of thoracotomy when the heart was removed for perfusion studies. Blood glucose control was determined from the percentage of glycosylated hemoglobin. Serum insulin and leptin concentrations were measured using ultrasensitive rat insulin (DRG Instruments GmbH) and rat leptin (Assay Designs) enzyme immunoassays. Serum nonesterified free fatty acid concentrations were measured using an enzymatic colorimetric assay (Roche).

Cardiomyocyte Necrosis and Apoptosis

From a longitudinal section 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 (please see the online Data Supplement for details). The degree of apoptosis was quantified as described previously 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; please see the online Data 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 described previously on tissue from the lateral wall of the left ventricle collected from rats that had not undergone hemodynamic assessment (please see the online Data Supplement for details).

Myocardial Collagen

Samples of LV tissue were weighed and stored at −70°C before tissue analysis. Myocardial hydroxyproline concentration (HPRO) was determined after acid (HCl) hydrolysis. Myocardial collagen was also extracted and digested with cyanogen bromide and subjected to acid hydrolysis and HPRO determination. The amounts of noncross-linked (soluble) and cross-linked (insoluble) collagen in the myocardium were ascertained on the basis of the solubility of myocardial collagen to cyanogen bromide digestion.

Myocyte Isolation and Assessment of Cell Morphometry

Cardiomyocyte morphology was determined on isolated cardiomyocytes using image analysis. Cell isolation was performed as described previously (please see the online Data Supplement for details), and the technique for image analysis is outlined in the online Data Supplement.

Data Analysis

Regression analysis was used to determine the lines of best fit for the cardiac function relations. All of the data are presented as mean±SEM. Comparisons between groups were made with a 2-way ANOVA.

Results

Characteristics of the Obesity Model

At the initiation of the study, WKY rats were heavier than SHRs, 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 SHRs as compared with 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 glycosylated hemoglobin measurements, was unchanged in either SHRs or WKY rats receiving the experimental diet (Table 1). Consistent with a reduced body size and, hence, adipose tissue content in SHRs, serum leptin concentrations were reduced in SHRs (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 SHRs but not in WKY rats (Table 1).

Cardiac Weight and Cardiomyocyte Size

Despite SHRs having increased systolic BP values, because SHRs were considerably smaller than WKY rats, SHRs receiving the control diet had similar absolute heart and LV weights and cardiomyocyte length and width values as compared with WKY rats (Table 1). However, when normalized for body weight differences, SHRs had marked increases in LV weights as compared with WKY rats (Table 1). The experimental diet increased LV weight and cardiomyocyte width but not length in SHRs (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: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).
LV Pump Function

The SHRs receiving the control diet had a greater LV endocardial fractional shortening (FSend; Figure 1, right) and a left shift in the LV systolic pressure-volume relationship (Figure 1, left) as compared with WKY rats receiving the control diet. However, pump function, as assessed using the load and heart rate–independent index of function, LV end systolic elastance (Ees; Figure 1, middle), was unchanged in SHRs as compared with WKY rats receiving a control diet. The experimental diet given to SHRs resulted in a decrease in LV systolic chamber function, as indicated by a decline in 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).

**Table 1. Effect of an Obesity-Inducing Diet (Diet) on Morphological, Blood, and Hemodynamic Characteristics in SHRs and WKY Rats**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WKY Control (n=10)</th>
<th>WKY Diet (n=9)</th>
<th>SHR Control (n=10)</th>
<th>SHR Diet (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight, g</td>
<td>484±9</td>
<td>527±8†</td>
<td>362±5‡</td>
<td>400±6‡</td>
</tr>
<tr>
<td>Tibial length, cm</td>
<td>4.61±0.12</td>
<td>4.39±0.12</td>
<td>4.68±0.17</td>
<td>4.37±0.12</td>
</tr>
<tr>
<td>Body weight/tibial length, /10</td>
<td>10.6±0.3</td>
<td>12.2±0.5†</td>
<td>7.6±0.3‡</td>
<td>8.7±0.2‡</td>
</tr>
<tr>
<td>Visceral fat, g</td>
<td>19.4±1.5</td>
<td>29.4±1.3†</td>
<td>9.6±0.7‡</td>
<td>17.2±0.7‡</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>124±4</td>
<td>122±4</td>
<td>184±5‡</td>
<td>166±3‡</td>
</tr>
<tr>
<td>Glycated hemoglobin, %</td>
<td>4.61±0.22</td>
<td>4.42±0.06</td>
<td>4.78±0.08</td>
<td>4.66±0.09</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.41±0.15</td>
<td>1.52±0.07</td>
<td>1.52±0.07</td>
<td>1.70±0.23§</td>
</tr>
<tr>
<td>LV weight, g</td>
<td>1.17±0.15</td>
<td>1.23±0.05</td>
<td>1.22±0.08</td>
<td>1.34±0.14§</td>
</tr>
<tr>
<td>LV weight/BW, ×100</td>
<td>2.40±0.003</td>
<td>2.33±0.004</td>
<td>3.41±0.004‡</td>
<td>3.55±0.002‡</td>
</tr>
<tr>
<td>LV weight/tibial length, ×10</td>
<td>2.53±0.10</td>
<td>2.82±0.11</td>
<td>2.63±0.008</td>
<td>3.08±0.15*‡</td>
</tr>
<tr>
<td>Cardiomyocyte width, μm</td>
<td>17.3±0.4</td>
<td>16.0±0.4</td>
<td>17.1±0.5</td>
<td>19.0±0.7†‡</td>
</tr>
<tr>
<td>Cardiomyocyte length, μm</td>
<td>71.7±1.1</td>
<td>73.0±2.1</td>
<td>76.9±2.1</td>
<td>73.9±2.8</td>
</tr>
<tr>
<td>LV EDD, mm</td>
<td>7.51±0.16</td>
<td>7.68±0.27</td>
<td>5.54±0.27†</td>
<td>6.64±0.27</td>
</tr>
<tr>
<td>LV ESD, mm</td>
<td>4.40±0.24</td>
<td>3.85±0.20</td>
<td>1.66±0.19</td>
<td></td>
</tr>
<tr>
<td>LV posterior wall thickness, mm</td>
<td>2.00±0.10</td>
<td>2.16±0.13</td>
<td>2.98±0.20</td>
<td>2.56±0.10</td>
</tr>
<tr>
<td>LV relative wall thickness, mm</td>
<td>0.54±0.04</td>
<td>0.57±0.05</td>
<td>1.04±0.11†</td>
<td>0.79±0.06</td>
</tr>
<tr>
<td>Serum leptin, pg/mL</td>
<td>114±128</td>
<td>1040±155</td>
<td>531±85∥</td>
<td>1014±81</td>
</tr>
<tr>
<td>Serum insulin, μg/L</td>
<td>0.35±0.03</td>
<td>0.34±0.04</td>
<td>0.38±0.06</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>Serum FFA, μmol/mL</td>
<td>0.38±0.05</td>
<td>0.41±0.04</td>
<td>0.40±0.05</td>
<td>0.51±0.04</td>
</tr>
</tbody>
</table>

EDD indicates end diastolic diameter; ESD, end systolic diameter; FFA, nonesterified free fatty acids.

*P<0.05 vs SHR control diet group or WKY control diet group.
†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.

Figure 1. Effect of an obesity-inducing diet (Diet) on LV pump function in SHRs and WKY rats, as determined ex vivo from LV systolic end pressure-volume relations (left) and the slope of these relations (LV Ees; middle) and in vivo from LV FSend measurements (right). *P<0.05 for an interaction between the presence of hypertension (SHR vs WKY) and diet category.
LV Intrinsic Myocardial Systolic Function

The SHRs and WKY rats receiving the control diet had a similar intrinsic myocardial systolic function (systolic stress-strain relationships and LV myocardial end systolic elastance were comparable; Figure 2). The experimental diet given to SHRs 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 myocardial end systolic elastance; Figure 2). However, the experimental diet failed to modify intrinsic myocardial systolic function in WKY rats (Figure 2).

LV Chamber Remodeling

Although SHRs had a left shift in the LV diastolic pressure-volume relationship (Figure 3, top left) and a decrease in LV end diastolic volume (Figure 3, top left), after normalizing LV volumes to 100-g body weight in SHRs and WKY rats. Bottom left, *P<0.005, SHR control vs other groups. Bottom right, *P<0.005 for an interaction between the presence of hypertension (SHR vs WKY) and diet category.

Figure 2. Effect of an obesity-inducing diet (Diet) on LV intrinsic myocardial systolic function in SHRs and WKY rats as determined ex vivo from LV systolic stress-strain relations (left) and the slope of these relations (LV myocardial end systolic elastance; LV myocardial end systolic elastance; right). *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 LV end diastolic pressure-volume relations (top) and the volume intercept (LV Vo) of these relations (bottom), before (left) and after (right) normalizing LV volumes to 100-g body weight in SHRs and WKY rats. Bottom left, *P<0.005, SHR control vs other groups. Bottom right, *P<0.005 for an interaction between the presence of hypertension (SHR vs WKY) and diet category.
volume at 0 pressure (Figure 3, bottom left) and LV end diastolic diameter (Table 1), after adjustments of LV volumes for body weight. SHRs receiving the control diet had similar LV diastolic pressure-volume relations and LV volume at 0 pressure values as WKY rats (Figure 3, right). Despite having similar LV weights as age-matched WKY rats, SHRs had increased LV end diastolic posterior and relative wall thickness values (Table 1). The experimental diet given to SHRs resulted in a right shift in the LV diastolic pressure-volume relationships (Figure 3, top), an increase in LV volume at 0 pressure (Figure 3, bottom), an increase in LV end diastolic diameter (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 volume at 0 pressure, LV end diastolic diameter, or LV posterior and relative wall thicknesses (Figure 3 and Table 1).

**Myocardial Apoptosis, Necrosis, and Interstitial Changes**

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

SHRs receiving a control diet had increased myocardial collagen concentrations, as indexed by HPRO measurements, as compared with 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 SHRs was attributed to an increase in myocardial collagen cross-linking, as detected by a decrease in myocardial collagen soluble to digestion by cyanogen bromide (Table 2). As a consequence of a decrease in myocardial collagen solubility in SHRs, the noncross-linked form of HPRO was unchanged in SHRs as compared with WKY rats receiving the control diet (Table 2). Administration of the experimental diet to either SHRs 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 SHRs. 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 SHRs occurred...
in the absence of further peripheral systolic BP changes or changes in blood glucose control (glycosylated hemoglobin). Dietary-induced pump dysfunction in SHRs was noted both in vivo (endocardial fractional shortening) and ex vivo using a load-independent assessment of chamber function (end systolic chamber elastance). In addition, dietary-induced pump dysfunction in SHRs 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 SHRs but not in WKY rats. However, dietary-induced pump dysfunction in SHRs 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, SHRs may have had myocardial collagen changes. The modest degree of generalized 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 SHRs.

Albeit that the results described in the present study were obtained in SHRs 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. In addition, whether the association between obesity and myocardial contractile disturbances in clinical studies reflects cause-and-effect relations is controversial, because weight loss produced either by lifestyle modification or gastric bypass does not influence LV myocardial systolic dysfunction. Although prospective studies in euglycemic animals indicate that obesity is associated with myocardial contractile disturbances, 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 SHRs 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 discernible adverse effects on the heart.

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

Although obesity-induced pump dysfunction in SHRs 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, and changes in arterial stiffness that influence central BP through early reflective waves are not closely emulated by peripheral BP values.

In agreement with previous studies conducted in euglycemic models of obesity in rodents, in the present study obesity was associated with increases in cardiomyocyte apoptosis but only in SHRs. In this regard, the excessive cardiomyocyte apoptosis in the SHRs 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. Because LVH is associated with cardiomyocyte apoptosis, the excessive apoptotic changes noted in obese SHRs but not in WKY rats in the present study may be attributed to an augmented LVH in SHRs. Alternatively, excessive cardiomyocyte apoptosis in obese SHRs 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, an effect that may result in ectopic lipid overload in cardiomyocytes (lipoaapoptosis). Nevertheless, other mechanisms may also play a role, including increases in circulating aldosterone concentrations noted to occur in obese SHRs.

Importantly, in the present study the degree of dietary-induced LV dilatation in SHRs exceeded that which could be explained by increases in body size, because marked right shifts in LV diastolic pressure-volume relations were noted in SHRs fed the experimental diet even after normalizing LV volumes to 100-g body weight. As demonstrated previously, cardiac dilatation is likely to contribute toward progressive pump dysfunction and heart failure in pressure-overload states. Whether obesity-induced cardiac dilatation noted in SHRs 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 collagenses (MMP-2), as opposed to changes in myocardial collagen concentrations or reductions in myocardial collagen cross-linking.

The limitations of the present study include the following. First, because 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 BP effects, per se. In this regard, further studies conducted in nongenetic 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 with 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, whereas 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, because 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 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.

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**Disclosures**

None.

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


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Abbreviated title: obesity and pump dysfunction

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Diets. The experimental diet consisted of weight/weight, 65% carbohydrates derived from maize and simple carbohydrates (sugar), 19% protein derived from soya and fish, and 16% fat derived from milk products and fish. In comparison, the control diet consisted of 60% carbohydrates derived from maize, 30% protein derived from soya and fish, and 10% fat derived from fish without milk products. The experimental diet consisted of weight/weight 7.8% saturated fats, 5.5% polyunsaturated fats and 2.6% monounsaturated fats. In contrast, the control diet consisted of weight/weight 1.9% saturated fats, 5.8% polyunsaturated fats and 2.6% monounsaturated fats.

Echocardiography. Left ventricular systolic function and chamber dimensions were determined in vivo as previously described 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. Left ventricular end-diastolic (LVEDD) and end-systolic (LVESD) internal diameter and end-diastolic (ED PWT) and end-systolic posterior wall thickness were measured and LV systolic chamber function (pump function) was determined from LV endocardial fractional shortening (LV FSEnd) = (LVEDD-LVESD)/LVEDD x 100. Left ventricular remodeling was determined from LV EDD and ED PWT measurements as well as LV relative wall thickness values calculated from ED PWT/(0.5 x LVEDD).

Isolated, perfused heart preparations. Briefly, hearts were perfused retrogradely via the aorta at a constant flow (12 ml.min\(^{-1}\).g wet heart weight) with 37°C physiological saline solution consisting of (in mM) 118.0 NaCl, 4.7 KCl, 2.5 CaCl\(_2\), 25.0 NaHCO\(_3\), 1.2 KH\(_2\)PO\(_4\), 1.2 MgSO\(_4\) and 10.0 glucose with a pH of 7.4 and saturated with 95% O\(_2\) and 5% CO\(_2\). To achieve equivalent flow rates per gram of tissue, the perfusion pressures in the groups were SHR-control diet=93±4 mm Hg, SHR-experimental diet=104±4 mm Hg, WKY-control diet=85±5 mm Hg, and WKY-experimental diet=88±4 mm Hg (p<0.05 for SHR-experimental diet vs WKY groups). Hearts were paced at 360 beats.min\(^{-1}\) with platinum electrodes attached to the left atrium and the apex of the heart. Left ventricular pressures were determined at multiple increments in volume, using a thin-walled latex balloon with a known wall volume, coupled to a Statham P23 pressure transducer and a micromanipulator. Load-independent LV systolic chamber performance (a measure of systolic pump function) was determined from the slope (Ees) of the linear portion of the LV peak systolic pressure-volume relation. Intrinsic myocardial systolic performance (a load-independent measure of intrinsic myocardial contractility) was assessed from the slope (En) of the systolic stress (\(\sigma\))-strain relation. Systolic \(\sigma\) and strain were calculated using previously described formulae, assuming a thick-walled, spherical model of LV geometry. LV remodeling was assessed in isolated, perfused heart preparations from the volume intercept (V\(_0\)) of the LV diastolic P-V relation.

Cardiomyocyte necrosis. Left ventricular tissue was stored in formalin, processed routinely for light microscopy and 5 \(\mu\)m-thick sections of the long axis circumference cut through the full thickness of the LV wall. Ten slices were
obtained at 1-mm intervals and stained with van Gieson’s stain. After staining, a pathological grade was assigned, where 0 indicates no damage; 1 and 2, patchy fibrosis in less than or more than 20% of the field respectively; 3 and 4, diffuse contiguous subendocardial fibrosis in less than or more than 50% of the field respectively and 5 and 6, full thickness fibrosis in less than or more than 50% of the field respectively. All data were obtained by a single observer unaware of the identity of the animal from which the sections were derived.

Cardiomyocyte apoptosis. Nuclear deoxyribonucleic acid (DNA) fragments in the tissue sections were detected using a non-radioactive in situ apoptotic cell death detection kit (DeadEnd™ Colorimetric TUNEL system, Promega, Madison, WI, USA), where terminal deoxynucleotidyl transferase (TdT) was used to incorporate biotinylated nucleotide at the 3'-OH DNA ends. The number of apoptotic cardiomyocyte nuclei and the total number of cardiomyocyte nuclei (haematoxylin and eosin stain) in each slide were counted on ten evenly spaced fields from the apex to the base using a computer-based image acquisition and analysis system at 400 times magnification (Axiovision 3, Carl Zeiss, Gottingen, Germany). Apoptotic cardiomyocyte nuclei were expressed as a percentage of the total number of cardiomyocyte nuclei. All data were obtained by a single observer unaware of the identity of the animal from which the sections were derived.

Myocardial matrix metalloproteinase activity. Tissue samples were frozen in liquid nitrogen within 5 minutes of removing hearts from the thoracic cavity and then stored at -70°C until analysis. In order to determine the relative activity of MMP-2 in each sample, 20 μg of protein was loaded into each well of a 10% polyacrylamide gel containing 1mg.ml⁻¹ of type A gelatin. The proteins were separated electrophoretically over 1.5 hours at 30 mA. A single standard of rat MMP-2 (Sigma, purity >95% by SDS-PAGE visualized by silver staining) was included on each gel to locate the MMP-2 bands. The gels were then incubated overnight in substrate buffer (Tris 50 mM pH 8, CaCl₂ 5 mM) to allow degradation of gelatin and the gels were stained for protein with Coomassie blue dye. Gels were scanned using a flat bed transmission scanner (Cano Scan 4200 F, Cannon Solutions, China) and images were inverted and the density of the MMP band analyzed compared to a standard extract MMP sample, using digital densitometry with Gene Tools software (Syngene, Cambridge, UK).

Myocyte isolation and assessment of cell morphometry. Samples of the lateral wall of the LV were minced and placed in a tissue culture flask containing 10 ml of incubation solution (15 mg Collagenase Type 2 [Worthington Biochemical Corporation, Lakewood NJ, USA] [317 U/mg], 14mg of Hyaluronidase [Worthington Biochemical Corporation, Lakewood NJ, USA] [581 U/mg], 125 mg of bovine serum albumin [Fraction V, Sigma Chemical Co., St. Louis, MO] and 0.0227 ml of 110 mM CaCl₂ dissolved in 50 ml of calcium free physiological saline solution [PSS]). The PSS consisted of NaCl 120 mmol, KCl 10 mmol, KH₂PO₄ 1.2 mmol, MgCl₂ 2.6 mmol, glucose 10 mmol, taurine 20 mmol, pyruvate 6.2 mmol and HEPES (4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid hemisodium salt) 4.8 mmol dissolved in 100 ml of distilled water (all chemicals obtained from Sigma Chemical Co., St. Louis, MO), and
pH=7.4. Tissue samples were placed in a preheated (37 °C) oscillating water bath (60 cycles per minute) for two periods of 10 minutes each and for a further period of 15 minutes (120 cycles per minute). Throughout the oscillation procedure the incubation solution containing the tissue was gassed with 95% O₂ and 5% CO₂. The incubation solution containing digested tissue and tissue precipitants was flushed through a 250-μm nylon mesh using ~10 ml of 0.045 ml of 110 mM CaCl₂ and 250 mg of bovine serum albumin.

For image analysis, cells were diluted in PSS containing 0.01% bromophenyl blue (The Coleman and Bell Co., USA). Using an inverted light microscope, cardiomyocytes were viewed at 400x magnification and images selected using a digital camera (Nikon Digital Sight DS-U1 & DS-5M, Nikon Corpora) based on the ability to see and count all sarcomere bands from one end of the cell to the other. Approximately 60-80% of the cells in the suspension were rod-shaped cardiac myocytes. Between 30 and 35 cells were selected from each LV tissue sample and using the program, Act2U (Ver. 1.70 Nikon Corporation, Japan), the dimensions of each cell were measured. Software was calibrated using a 0.01mm x 100 (1mm) graticule (Graticules Ltd, Kent, England). All images were stored for subsequent assessment of the length and width of each cell.

References