Basic Science and Experimental Studies

Sleeve Gastrectomy in Obese Zucker Rats Restores Cardiac Function and Geometry Toward a Lean Phenotype Independent of Weight Loss

TAMMY L. KINDEL, MD, PhD,[1] TOM FOSTER, BS,[1] LEANNE HARMANN, BA,[2] AND JENNIFER STRANDE, MD, PhD[2]

Milwaukee, Wisconsin

ABSTRACT

Objective: Bariatric surgery, including sleeve gastrectomy (SG), significantly improves cardiac geometry and function in patients with heart failure. In this study, we used the obese Zucker rat as an animal model of heart failure with preserved ejection fraction (HFpEF) to test the hypothesis that a SG will improve cardiac function independent of weight loss.
Methods and Results: Obese, male Zucker rats underwent SG, pair-fed sham, or ad-lib sham surgery. Lean Zucker rats also underwent ad-lib sham surgery. Echocardiograms were performed preoperatively and at 6 weeks postoperatively. Obese SG and obese pair-fed sham rats had similar body weights postoperatively. Obese SG and lean, ad-lib, sham rats had a significant increase in postoperative stroke volume, and left ventricular internal diameter in diastole and systole. SG preserved systolic function and significantly improved isovolumetric relaxation time (13.9 ± 2.4 to 11.1 ± 2.1 ms, $P = .02$) independent of weight loss.
Discussion: SG has a beneficial impact on both systolic and diastolic cardiac function in obese Zucker rats toward a lean phenotype independent of weight loss and caloric restriction. These findings may represent a weight-loss independent mechanism generated from the gastrointestinal tract that has the potential to improve diastolic dysfunction independent of obesity status and translate to patients with HFpEF. (*J Cardiac Fail 2019;25:372−379*)
Key Words: Heart failure with preserved ejection fraction, obesity, bariatric surgery, diastolic function.

Heart failure with preserved ejection fraction (HFpEF) is the most common form of heart failure and is closely associated with obesity.[1,2] HFpEF, in which the global left ventricular systolic function or ejection fraction (EF) is preserved (>50%), is a syndrome caused primarily from impaired cardiac relaxation and diastolic filling resulting in a decreased stroke volume and increased circulating blood volume. The consequential increased left ventricular pressures and decreased cardiac output results in the symptoms of heart failure. The cause for impaired diastolic function in obesity-associated HFpEF is unknown but likely related to systemic inflammation, an increased plasma volume, and sodium retention.[3] The risk of heart failure increases 5% for men and 7% for women with every increase in body mass index (BMI) point.[4] In patients with HFpEF, there is an increased risk of all-cause mortality and heart failure-related hospitalization with a BMI >35 kg/m^2.[5]

Bariatric surgery significantly improves both symptoms of heart failure and cardiac geometry and function including decreased left ventricular hypertrophy and dilation along with improved systolic function.[6-8] There is an almost universal improvement in diastolic function after bariatric surgery because of decreased relaxation time of the left ventricle.[6] It is possible that the benefits of bariatric surgery are related to the significant postoperative weight loss alone. Unfortunately, it has been very difficult to study the effect of significant weight loss outside of bariatric surgery

because of the exceptionally high failure rate of dieting and pharmacotherapy in this patient population. Alexander et al[9] studied 9 severely obese patients who lost significant weight with dieting and had decreases in systemic arterial pressure, stroke volume, and cardiac output without improvements in myocardial hypertrophy and ventricular compliance. More modest weight loss through dieting decreases left ventricular mass with variable changes in diastolic function.[10,11] The lack of efficacy of dieting on cardiac function compared with bariatric surgery suggests a weight-loss independent mechanism initiated by altering the gastrointestinal tract with bariatric surgery, which improves postoperative cardiac function in patients with heart failure.

Sleeve gastrectomy (SG) is a commonly performed bariatric surgery in which the greater curvature of the stomach is removed. We have previously found that 44% of high-fat fed Wistar rats that underwent a SG maintained normal systolic function without an effect on diastolic function, compared to 0% in control rats[12]. The variable improvement in cardiac function was weight-loss independent suggesting a potential novel, metabolic role of bariatric surgery to beneficially alter the entero-cardiac axis and improve cardiac function. Although the Wistar rat develops obesity with a high-fat diet, it is an insulin-sensitive model; therefore, we hypothesized that a more robust rodent model of cardiac dysfunction encompassing components of obesity-associated HFpEF including insulin resistance, cardiac hypertrophy and diastolic impairment would enhance the impact of a SG on cardiac dysfunction. In this study, we used the obese Zucker rat as a model of metabolic syndrome and cardiac dysfunction to test the hypothesis that SG will improve cardiac function independent of weight loss.

Methods

Study Design

Zucker rats were used as an outbred model of obesity and metabolic syndrome with insulin resistance and dyslipidemia to study the effect of SG on cardiac function. Obese (strain 185) or lean (strain 186) male, Zucker rats were purchased at 5 weeks of age (Charles River Laboratories). All rats were housed in pairs prior to surgery and started on Purina Diet 5008 at 8 weeks of age. A preoperative echocardiogram was performed at 12 weeks of age on all rats. Zucker rats were stratified into 4 different surgical groups: obese SG, obese pair-fed sham, obese ad-lib sham, and lean ad-lib sham surgery. Obese rats were stratified into the 3 groups matched for ejection fraction and body weight. After surgery, animals were individually caged for the measurement of body weight and food intake and resumed Purina diet 5008 72 hours after surgery until study completion. All groups underwent a postoperative echocardiogram at 6 weeks. After 5−7 days of recovery, animals were euthanized with collection of blood and ex vivo heart weights. This study and all procedures were approved by the Medical College of Wisconsin's Institutional Animal Care and Use Committee.

Surgical Procedures and Pair-Feeding

Solid food was replaced with Vanilla Ensure (Abbott, Lake Forest, IL) for postoperative recovery starting 24 hours prior to surgery and for 72 hours after surgery. All animals were fasted overnight but had ad libitum access to water. SG and pair-fed sham surgeries were performed as previously described including preoperative antibiotic prophylaxis with enrofloxacin (10 mg/kg subcutaneously), buprenorphine SR for perioperative pain control, and subcutaneous saline bolus (20 cc/kg) on postoperative day 0 and 1.[12] In brief, after division of the greater curve fibrofatty tissue, the stomach was opened on the lateral segment removing any additional stomach contents, and then the stomach divided with an endoscopic stapling device (Endopath ETS-Flex, 45 mm white load; Ethicon, Cincinnati, OH) to include all of the non-glandular stomach and glandular lateral fundus. For sham animals, the same procedure was followed except the stomach was not divided. SG induces a decrease in food intake. Pair-feeding was performed in one obese, sham Zucker group to create rats that would be calorically-restricted to the exact same degree over time as obese SG rats. Obese, pair-fed sham rats were given the same amount of daily food consumed by the obese SG rats for the same postoperative day throughout the study period. For example, if the average food intake of obese SG rats was 15g on postoperative day #15, all obese, pair-fed sham rats would receive only 15 g of food for their respective postoperative day 15.

Cardiac Function Analysis

Rodent echocardiograms were performed in anesthetized (2% isoflurane) rats at 13 weeks of age (1 week preoperatively) and at 6 weeks postoperatively. Measurements and data analyses were performed as previously published by a small animal echocardiogram technician blinded to the study groups.[12,13] Animals were studied in the left lateral decubitus position with a commercially available echocardiographic system (Vivid 7, General Electric, with an 11-MHz M12-L linear array transducer, GE Healthcare, Waukesha, WI). Echocardiograms were performed from the cardiac short axis of the left ventricle at the papillary muscle level, using the anatomical M-mode feature of the Vivid 7 echo. A M-mode display was generated from raw data 2D images with the line selected passing through the anterior and inferior segments. Stroke volume was measured by subtracting left ventricular end systolic volume from end diastolic volume. EF was measured using the formula: EF = stroke volume/end diastolic volume × 100. The left ventricular mass was derived from the anteroseptal thickness and inferolateral thickness using the formula: left ventricular mass = 0.8 + (1.04[(inferolateral thickness + left ventricular internal diameter in diastole + anteroseptal thickness)^3 − (left ventricular internal diameter in

diastole)^3] + 0.6. Diastolic function was measured by tissue Doppler including the isovolumetric relaxation time, E wave, and mitral annulus (e′) velocity. Three consecutive heart beats were measured and the average used for analysis.

Hormone Analysis

Blood was collected in EDTA collecting tubes from the inferior vena cava at the time of euthanasia at 7 weeks postoperatively. Fasting glucose was measured immediately in duplicate using a handheld glucometer. Samples were kept on ice and then spun for 10 minutes at 4000 g with plasma collected for the measurement of insulin, cholesterol, and triglyceride. Insulin concentrations were determined using a commercially available rat, sandwich enzyme-linked immunosorbent assay kit (MilliporeSigma, Burlington, MA). Cholesterol and triglycerides were measured using an enzymatic detection method by an ACE Alera clinical chemistry system (Alfa Wassermann, West Caldwell, NJ). Free fatty acids were measured in duplicate using a commercially available Free Fatty Acid Quantification kit (Sigma-Aldrich, St. Louis, MO).

Histology

Under general anesthesia, the sternum was divided and the heart excised in total. The aortic root was infused with up to 30 mL of a 1.5% solution of potassium chloride in phosphate buffered solution to arrest the heart in diastole. After trimming any excess attached tissue or epicardial fat, the heart was blotted and weighed. The left mid ventricle was sharply sectioned and placed immediately in OTC medium, frozen in dry ice, and stored at −80°C until sectioned. Tissue sections were stained for hematoxylin and eosin for cardiomyocyte size, Masson trichrome stain for interstitial fibrosis, and Oil Red O for lipid deposition. Cardiomyocyte size was measured from each animal as a total of 50 random cells per heart section to give a mean size for each individual. Slides were scanned at 40× magnification using the Nanozoomer 2.0-HT (Hamamatsu, Japan) digital

slide scanning system (Children's Research Institute's Imaging Core). High-resolution whole-slide scanned images were imported into Visiopharm software (Denmark) and then using the imager, module collagen-positive regions were identified by thresholding the specific image channel filter compared to the rest of the tissue (sum of muscle, nuclear, and collagen region without any interstitial space). All original images were processed with this preset threshold and linear Bayesian classification to generate a processed image. Total collagen-positive area per region-of-interest was measured in micrometers and represented as a percentage of the total tissue area.

Statistics

All values are presented as the mean ± standard deviation. Comparisons of results between preoperative and postoperative time points for each group was performed as a student's t test with multiple groups at the same time point as a one-way ANOVA with post hoc analysis performed for pairwise multiple comparisons using the Holm−Sidak method with equal variance and assumed normality. In cases of unequal variance, a Kruskal−Wallis one-way ANOVA of ranks was performed with post hoc pairwise comparisons by Dunn's methods. Values were determined statistically significant if $P < .05$. Sigmastat v4.0 was used for all statistical analysis.

Results

SG Significantly Reduces Body Weight Compared to Sham Surgery

Obese or lean strains of Zucker rats were started on Purina diet 5008 at 8 weeks of age. Once 13 weeks old, the obese rats underwent SG or sham surgery after matching for EF and body weight. All lean rats underwent sham surgery at 13 weeks of age. Our long-term survival rate for obese SG rats was 92% (n = 11), 75% for obese, pair-fed, sham rats (n = 6) and 100% for obese, ad-lib sham (n = 8) and lean sham rats (n = 8). There was no significant difference in body weights
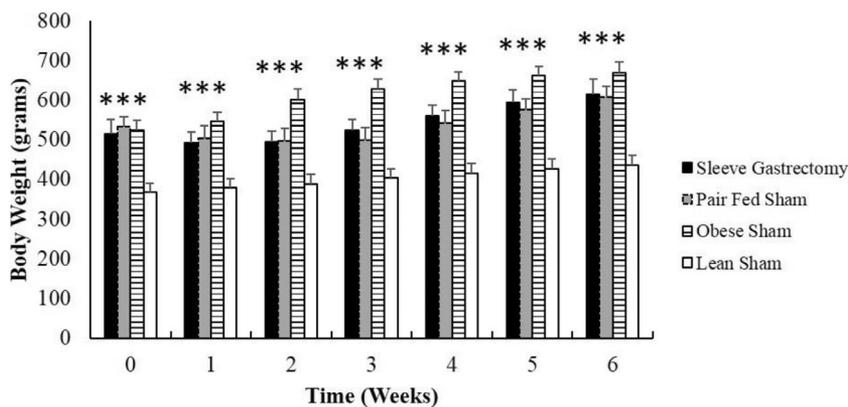

Fig. 1. The change in postoperative body weight over time after SG or sham surgery (performed at time 0). *$P < .05$ for the designated surgical group compared to lean sham Zucker rats at each time point. Data are presented as the mean ± standard deviation.
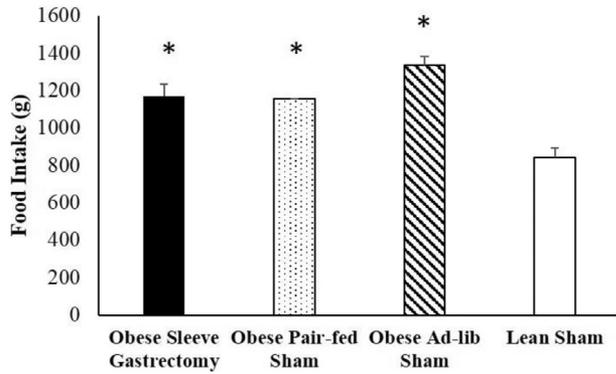
Fig. 2. The cumulative food intake of each surgical group for 6 postoperative weeks (n = 6−11/group). Food intake of the pair-fed sham group was matched to the daily intake of the SG group. *P < .05 for the designated surgical group compared to lean sham Zucker rats. Data are presented as the mean ± standard deviation.

of the 3 obese Zucker rat groups preoperatively, obese SG 515.7 ± 36.4 g, obese, pair-fed sham 532.5 ± 27.0 g, and obese, ad-lib sham 525.1 ± 23.4 g. All obese Zucker rat groups weighed significantly more than the lean sham group for the 6 weeks postoperatively (Fig. 1). There was no difference in postoperative body weights between obese SG and obese, pair-fed, sham groups at any time point postoperatively with the obese SG group weighing 615.4 ± 37.7 g at 6 weeks postoperatively compared to the obese, pair-fed, sham group weighing 607.8 ± 28.4 g (P = .63). Because of a significant reduction in postoperative food intake compared to obese, ad-lib sham rats, obese SG rats and obese, pair-fed, sham rats weighed significantly less than obese, ad-lib sham rats at every week postoperatively. Obese SG and obese, pair-fed, sham rats ate a similar amount of cumulative food over the 6-week postoperative period (obese SG 1170.7 ± 62.8 g vs obese, pair-fed sham 1158 g, P = .61). However, as shown in Figure 2, all obese groups ate more than the lean sham group over the postoperative study period.

SG in Obese Zucker Rats Preserves Systolic Function With Increased Stroke Volume and Preserved Cardiac Mass

There are multiple inherent differences in cardiac function between the outbred obese and lean Zucker rat by echocardiogram performed pre-operatively at 11 weeks of age (Table 1). The obese Zucker rat has significantly increased left ventricular wall thickness, internal left ventricular diameter in diastole and systole, and volumes (end systolic, end diastolic, and stroke volume) with preserved fractional shortening and EF compared to lean Zucker rats. Obese Zucker rats also had an increased left ventricular mass but a significant decrease in left ventricular mass compared to body weight with impaired diastolic function as indicated by a significantly increased isovolumetric relaxation time of 14.2 ± 8.2 ms compared to lean Zucker rats with an isovolumetric relaxation time of 10.3 ± 1.9 ms, P < .001.

Table 1. Difference in Preoperative Cardiac Function Between Obese and Lean Zucker Rats

	Lean Zucker (n = 8)	Obese Zucker (n = 25)	P Value
BW (g)	355.8 ± 22.6	521.4 ± 28.2	<.001*
IVSd (cm)	0.17 ± 0.02	0.19 ± 0.02	.10
LVIDd (cm)	0.76 ± 0.02	0.85 ±0.06	<.001*
LVIDs (cm)	0.34 ± 0.05	0.42 ± 0.09	.03*
LVPWd (cm)	0.16 ± 0.02	0.18 ± 0.02	.05*
EDV (mL)	0.98 ± 0.09	1.32 ± 0.24	<.001*
ESV (mL)	0.11 ± 0.05	0.20 ± 0.11	.03*
EF (%)	89.3 ± 4.2	85.4 ± 6.7	.13
FS (%)	55.2 ± 5.7	50.9 ±8.3	.18
SV (mL)	0.87 ± 0.06	1.12 ± 0.18	<.001*
LVM (g)	1.33 ± 0.08	1.58 ± 0.12	<.001*
%LVM/BW	0.37 ± 0.03	0.30 ± 0.02	<.001*
IVRT (ms)	10.3 ± 1.9	14.2 ± 8.2	<.001*
E (m/s)	1.35 ± 0.11	1.35 ± 0.16	.94
Decel time (ms)	47.8 ± 4.3	46.7 ± 8.2	.71
e′ (m/s)	0.10 ± 0.03	0.08 ± 0.02	.05*
E/e′	14.8 ± 5.0	17.8 ± 4.3	.09

Data are presented as the mean ± standard deviation.
IVSd, interventricular septum in diastole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVPWd, left ventricular posterior wall in diastole; EDV, end diastolic volume; ESV, end systolic volume; EF, ejection fraction; FS, fractional shortening; SV, stroke volume; LVM, left ventricle mass; BW, body weight; IVRT, isovolumetric relaxation time; E, E wave; e′, mitral annulus velocity.
*Statistical significance determined at P < .05 comparing lean versus obese Zucker rats.

As shown in Table 2, echocardiograms performed preoperatively were compared for measure of both systolic and diastolic function for each group to 6 week postoperative echocardiograms. Obese SG and lean Zucker rats were the only groups to significantly increase stroke volume from pre-op (obese SG; 1.04 ± 0.16 to 1.25 ± 0.25 mL, P = .03; lean: 0.87 ± 0.06 vs 0.97 ±0.09 mL, P = .03). For both obese SG and lean Zucker groups, there was also a significant increase in left ventricular internal diameter in both systole and diastole postoperatively. Obese, pair-fed and obese, ad-lib sham rats had no difference in stroke volume, left ventricular internal diameter in diastole or systole postoperatively compared to pre-operative values. Obese SG rats were the only group to maintain percentage left ventricular mass to body weight with 0.30 ± 0.02% preoperatively and 0.29 ± 0.04% postoperatively (P = .25), with the 3 remaining groups, including lean Zucker rats, displaying a significant decrease. Systolic function, as measured by both fractional shortening or EF as shown in Figure 3, was preserved postoperatively only in obese SG rats with a preoperative EF of 83.8 ± 7.8% and postoperative EF of 79.5 ± 6.3%, P = .17. Lean Zucker rats also had a decline in EF with an EF of 89.3 ± 4.2% preoperatively and 82.0 ± 7.3% postoperatively, P = .03. Although there were no echocardiogram differences between obese SG and obese, pair-fed, sham rats pre-operatively, obese SG rats had a significantly higher left ventricular mass and percentage left ventricular mass to body weight postoperatively compared to obese, pair-fed rats (Table 2). These differences in cardiac parameters exist between obese SG and obese, pair-fed, sham rats

Table 2. Effect of SG or Sham Surgery on Cardiac Structure and Left Ventricular Systolic Function at 2 Weeks Preoperatively and 6 Weeks Postoperatively in Zucker Rats

	IVSd (cm)	LVIDd (cm)	LVIDs (cm)	LVPWd (cm)	EDV (mL)	ESV (mL)	SV (mL)	LVM (g)	%LVM/BW
Obese SG (n = 11)									
Pre	0.19	0.83	0.43	0.18	1.26	0.22	1.04	1.57	0.30
Post	0.20	0.9*	0.58*	0.20*	1.47	0.33	1.25*	1.79*†	0.29†
Obese pair-fed sham (n = 6)									
Pre	0.20	0.86	0.44	0.18	1.35	0.22	1.13	1.63	0.32
Post	0.19	0.86	0.51	0.17	1.36	0.34	1.03	1.57	0.25*
Obese ad-lib sham (n = 8)									
Pre	0.17	0.86	0.39	0.18	1.37	0.16	1.21	1.54	0.29
Post	0.20*	0.85	0.51	0.21*	1.35	0.37	0.98	1.74*	0.26*
Lean ad-lib sham (n = 8)									
Pre	0.17	0.76	0.34	0.16	0.98	0.11	0.87	1.33	0.37
Post	0.18	0.81*	0.44*	0.17	1.17*	0.21*	0.97*	1.44*	0.33*

Data are presented as the mean. Statistical significance determined at $P < .05$ comparing preoperative versus postoperative values within groups (*) or comparing sleeve gastrectomy versus pair-fed (†) for the same time point.

IVSd, interventricular septum in diastole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVPWd, left ventricular posterior wall in diastole; EDV, end diastolic volume; ESV, end systolic volume; SV, stroke volume; LVM, left ventricle mass; BW, body weight.

despite the 2 groups being similar in postoperative age, diet, body weight, and food intake.

At the time of euthanasia, intact hearts were excised, weighed, and analyzed relative to body weight. Compared to lean sham Zucker rats, both obese, ad-lib and obese, pair-fed, sham rats had significantly smaller heart weight to body weight ratios. Obese SG rats had similar percentage heart weight to body weight compared to lean sham rats ($0.23 \pm 0.03\%$ vs $0.25 \pm 0.02\%$, $P = .07$; Fig. 4). There was no difference in cardiomyocyte area between lean ($391.8 \pm 42.0 \ \mu m^2$) and obese SG ($435.7 \pm 2.9 \ \mu m^2$), obese ad-lib sham ($555.4 \pm 8.9 \ \mu m^2$), and obese, pair-fed, sham groups ($363.5 \pm 45.7 \ \mu m^2$). There was no significant difference in the amount of interstitial collagen deposition by histology (Fig. 5) and quantification (Fig. 6) between lean and obese Zucker groups (obese SG: $6.28 \pm 0.55\%$, obese pair-fed sham: $6.71 \pm 1.53\%$, obese ad-lib sham: $6.34 \pm 0.52\%$, and

lean sham: $5.75 \pm 0.62\%$). There was also no difference in Oil Red O quantification between lean Zucker rats and obese SG, pair-fed sham, or ad-lib sham groups.

SG Significantly Improves Diastolic Relaxation Independent of Weight Loss

As shown in Table 3, obesity significantly impaired diastolic function at 6 weeks postoperatively in obese, ad-lib sham rats with a significant decrease in deceleration time, e′, and E/e′ compared to pre-operative values. Obese SG rats had a significant improvement in isovolumetric relaxation time from 13.9 ± 2.4 to 11.1 ± 2.1 ms, $P = .02$ that was not seen in any other group. This improvement postoperatively in obese SG rats was significantly different from obese, pair-fed, sham rats with an isovolumetric relaxation time of 16.0 ± 5.4 ms ($P = .02$).
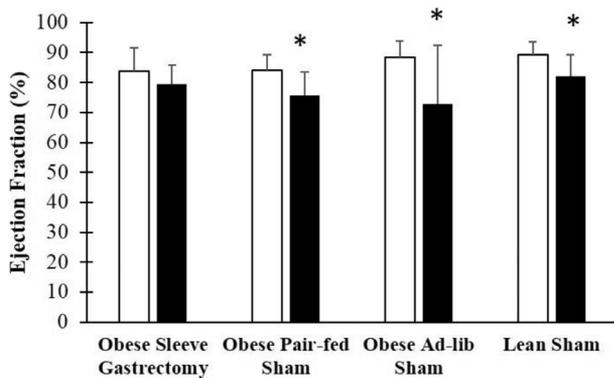

Fig. 3. Measurement of preoperative and 6-week postoperative EF by echocardiogram in all 4 surgical groups (n = 6−11/group). *$P < .05$ for the designated surgical group compared to preoperative values. Data are presented as the mean ± standard deviation.
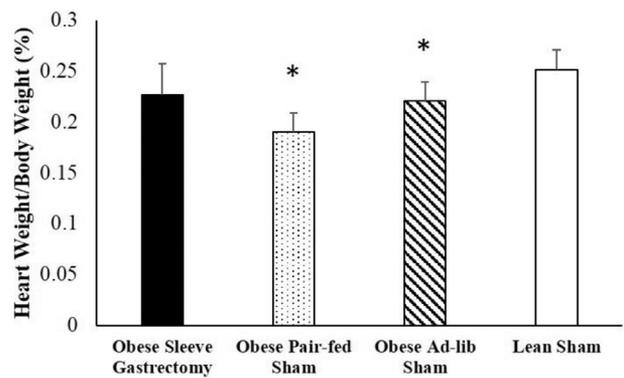

Fig. 4. Percentage of total heart weight to body weight (n = 6−10/group). *$P < 0.05$ comparing the designated surgical group compared to lean sham Zucker rats. Data are presented as the mean ± standard deviation.
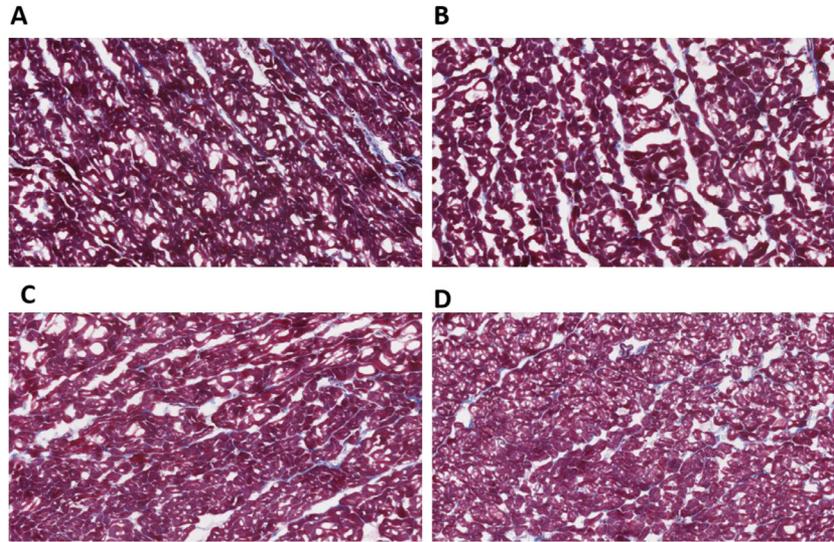
Fig. 5. Masson's trichrome staining (light blue color) of cardiac tissue sections (20×). Histopathologic analysis of cardiac tissue sections of the mid-left ventricle from the lean Zucker sham rats (A), obese SG Zucker rats (B), obese pair-fed Zucker sham rats (C), and obese ad-lib Zucker sham rats (D).

SG Decreases Serum Cholesterol Independent of Weight Loss

Prior to euthanasia at ~6−7 weeks postoperatively, blood was taken from the inferior vena cava and processed for plasma metrics of glucose and lipid homeostasis. As shown in Table 4, all obese Zucker groups (obese SG; obese, pair-fed sham; and obese, ad-lib sham) had significantly elevated non-fasting insulin concentrations compared to lean Zucker rats. There was no difference in glucose or free fatty acids concentrations among any of the obese groups compared to lean rats. SG uniquely restored cholesterol concentrations to that of lean Zucker sham rats ($P = .44$) where obese pair-fed sham ($P = .05$) and obese, ad-lib sham ($P = .01$) cholesterol concentrations were significantly higher than lean Zucker rats. Triglyceride concentrations were significantly elevated in obese, ad-lib,

sham rats compared to lean, sham rats (3164.4 ± 2992 vs 200.2 ± 58.7 mg/dL, $P = .05$).

Discussion

We have found that independent of weight loss, body weight, calorie restriction, and dietary changes, obese SG rats have a beneficial change in cardiac function toward a lean phenotype driven by improved left ventricular diastolic relaxation and filling, which preserves systolic function. Unlike obese, pair-fed sham and obese, ad-lib sham rats, the heart in obese SG and lean sham rats responds to weight-related expansion of plasma volume by dilating the ventricle. This is a normal cardiac compensatory response to increased volume.[2] In obese, pair-fed, sham and obese, ad-lib sham rats, there is inadequate dilation associated with impaired relaxation and marginal changes in end
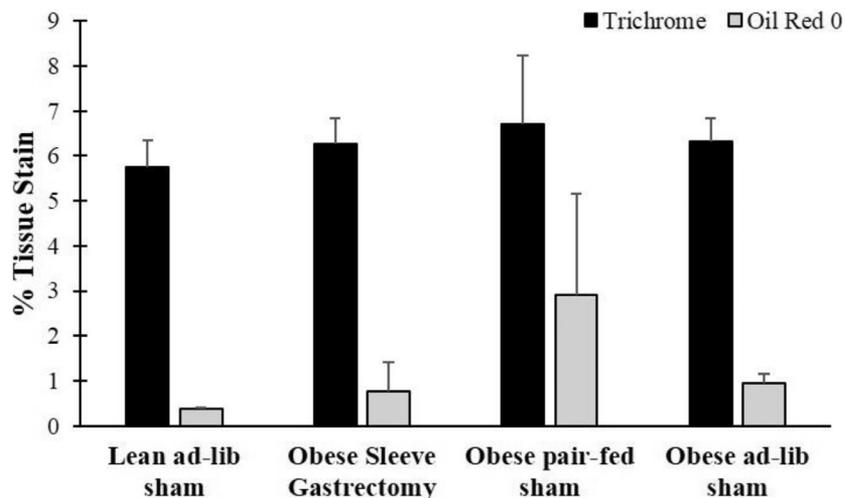

Fig. 6. Quantification of Masson's trichrome (black bars) and Oil Red O staining (grey bars) of left ventricular cardiac tissue sections.

Table 3. Effect of SG or Sham Surgery on Diastolic Function at 2 Weeks Preoperatively and 6 Weeks Postoperatively in Zucker Rats

	IVRT (ms)	E (m/s)	Decel time (ms)	e′ (m/s)	E/e′
Obese SG (n = 11)					
Pre	13.9 ± 2.4	1.29 ± 0.15	45.0 ± 9.2	0.07 ± 0.02	19.8 ± 4.4
Post	$11.1 \pm 2.1*†$	1.36 ± 0.11	44.1 ± 5.4	0.08 ± 0.02	18.2 ± 4.1
Obese pair-fed sham (n = 6)					
Pre	13.4 ± 4.2	1.40 ± 0.18	49.6 ± 10.1	0.08 ± 0.02	19.3 ± 4.5
Post	16.0 ± 5.4	1.26 ± 0.19	40.0 ± 5.2	0.07 ± 0.02	17.6 ± 6.4
Obese ad-lib sham (n = 8)					
Pre	15.3 ± 1.7	1.40 ± 0.14	46.9 ± 5.1	0.10 ± 0.02	14.5 ± 1.4
Post	14.2 ± 3.2	1.36 ± 0.14	$40.5 \pm 5.3*$	$0.08 \pm 0.02*$	$17.8 \pm 2.6*$
Lean ad-lib sham (n = 8)					
Pre	10.3 ± 1.9	1.35 ± 0.11	47.8 ± 4.3	0.1 ± 0.03	14.8 ± 5.0
Post	10.3 ± 1.9	1.33 ± 0.10	47.2 ± 8.6	0.08 ± 0.02	17.0 ± 3.2

Data are presented as the mean ± standard deviation. Statistical significance determined at $P < .05$ comparing preoperative versus postoperative values within groups (*) or comparing sleeve gastrectomy versus pair-fed (†) for the same time point. IVRT, isovolumetric relaxation time; E, E wave; e′, mitral annulus velocity.

diastolic volume despite an increased postoperative body weight with associated increased circulating blood volumes. With inappropriate cardiac accommodation to the volume demands of weight gain, the stroke volume of obese, pair-fed, sham and obese, ad-lib sham rats does not increase as it normally does in lean rats and as found after SG. Additional studies supporting this mechanism would include in vivo cardiac hemodynamics where we would expect to find elevated left ventricular filling pressures in both obese, pair-fed, sham, and obese ad-lib sham rats leading to symptoms of heart failure with reduced exercise capacity compared to obese SG and lean sham rats.

Similar to our previous publication with SG in Wistar rats, SG in obese Zucker rats preserved EF with a significant reduction in EF in all other sham models, including lean controls.[12] This finding suggests that SG protects against obesity- and age-related decline in EF in Zucker rats. There is a growing appreciation among bariatric surgeons and heart failure cardiologists that bariatric surgery has the potential to significantly improve systolic function. A recent meta-analysis found a significant improvement in EF after bariatric surgery.[8] Unlike control subjects, bariatric surgery significantly

improved EF, with improvements >10% significantly more likely to occur in bariatric surgery patients than control patients.[14] Further, systolic function after bariatric surgery is more likely in patients with nonischemic cardiomyopathy and long-standing obesity implying that the mechanism for systolic function improvement after bariatric surgery may be because of reversal of altered substrate utilization, cardiac insulin resistance, oxidative stress, and/or cardiac steatosis, all hallmarks of obesity-cardiomyopathy.[15,16]

Bariatric surgery in patients with preserved systolic function reduces left ventricular mass, wall thickness and blood pressure.[17] Cuspidi et al[18] published a systemic review of 23 studies consistently finding a reduction in left ventricular mass and wall thickness. A recent review of publications on the effect of bariatric surgery on diastolic function shows an almost universal increase in the peak early mitral flow velocity compared to late mitral flow velocity (E/A ratio) with significant reductions in isovolumetric relaxation times.[6] Mikhalkova[19] specifically studied the impact of bariatric surgery on cardiac function in patients with HFpEF. Bariatric surgery significantly decreased cardiac mass, left ventricular wall thickness, heart failure-related symptoms, and left ventricular relaxation.[19] Our animal study similarly found an improvement in left ventricular relaxation as implicated by the improvement in isovolumetric relaxation time in obese SG rats only and supports that diastolic function improvements after bariatric surgery may be mediated by more than just postsurgical weight loss, and instead be because of weight-loss independent changes mediated from the gastrointestinal tract.

Although the pathophysiology of HFpEF is incompletely understood with no effective treatment, there is a strong association between HFpEF and metabolic diseases such as obesity, hypercholesterolemia, hypertension, and type 2 diabetes mellitus. Beyond atherosclerosis, hypercholesterolemia can be directly cardiotoxic because of the induction of oxidative stress and proinflammatory cytokines.[20,21] In mice, a cholesterol diet increases serum cholesterol, peroxynitrite-mediated cardiac dysfunction and oxidative stress via increased expression of nicotinamide adenine dinucleotide phosphate oxidase.[22] Because we found only obese SG rats had a similar cholesterol concentration as lean sham rats, a reduction in systemic hypercholesterolemia and subsequent effect on oxidative stress may be a critical mediator for the beneficial effect of SG on diastolic function.

Murine models of HFpEF are inherently limited to capture all facets of clinical obesity-associated HFpEF, including

Table 4. Postoperative Nonfasting Plasma Parameters of Glucose and Lipid Metabolism

	Glucose (mg/dL)	Insulin (ng/mL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Free Fatty Acids (nmol/µL)
Obese SG	113.9 ± 13.6	$13.4 \pm 4.2*$	213.6 ± 42.6	725.1 ± 430.1	0.75 ± 0.8
Obese pair-fed sham	138.8 ± 37.4	$15.0 \pm 2.8*$	$370.6 \pm 100.3*$	1167.2 ± 633.9	0.78 ± 0.3
Obese ad-lib sham	176 ± 128.5	$16.0 \pm 4.4*$	$547.9 \pm 353.0*$	$3164.4 \pm 2992.0*$	1.83 ± 1.3
Lean sham	124 ± 20.5	3.7 ± 1.4	119.4 ± 25.5	200.2 ± 58.7	0.40 ± 0.4

Blood samples were drawn at 6 weeks postoperatively at the time of euthanasia. Data are mean ± SD. N = 5−11 for each group. Statistical significance determined at $p < .05$.
*Significance between the designated group compared with lean sham animals.

obesity, insulin resistance/type 2 diabetes, exercise intolerance, peripheral edema, increased left ventricular hypertrophy/mass, arterial stiffness, disturbed ventricular-arterial coupling, myocardial fibrosis, cardiomyocyte hypertrophy, decreased intra-myocardial capillary density, inappropriately depressed elevation of natriuretic peptides, and systemic inflammation[1]. However, Zucker rats are an excellent model of reproducible obesity, insulin resistance, metabolic abnormalities of hyperleptinemia, hypercholesterolemia, hypertriglyceridemia, but preserved glucose tolerance.[23,24] The Zucker model, as we have also confirmed in this study, has left ventricular hypertrophy with early diastolic dysfunction and, with age, progresses to moderate systolic dysfunction therefore representing a mixed model of heart failure with preserved and reduced EF. Our surgical model also does not parallel the post-bariatric process where patients have a dramatic change in postoperative diet to a protein enriched, very low fat and carbohydrate diet. Further, the SG rodent, as we and others have found, will generally lose weight initially but continue to gain weight postoperatively although not regaining weight back to obese, ad-lib sham controls (as is usually found with re-feeding after calorie restriction) limiting the comparison to clinical bariatric surgery.

Conclusions

This study is the first to find a weight-loss independent effect of SG on diastolic function with restoration of cardiac function and geometry toward a lean phenotype. We also found a preservation of both obesity and age-related systolic function in obese SG Zucker rats. These finding may represent a novel, potential surgical therapy for patients with HFpEF to reverse diastolic dysfunction and preserve systolic function independent of postoperative obesity status and weight loss.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

References

1. Valero-Munoz M, Backman W, Sam F. Murine models of heart failure with preserved ejection fraction: a "fishing expedition". JACC Basic Trans Sci 2017;2:770–89.
2. Packer M, Kitzman DW. Obesity-related heart failure with a preserved ejection fraction: the mechanistic rationale for combining inhibitors of aldosterone, neprilysin, and sodium-glucose co-transporter 2. JACC Heart Fail 2018;6:633–9.
3. Shah SJ, Kitzman DW, Borlaug BA, et al. Phenotype-specific treatment of heart failure with preserved ejection fraction: a multiorgan roadmap. Circulation 2016;134:73–90.
4. Kenchaiah S, Evans JC, Levy D, et al. Obesity and the risk of heart failure. N Engl J Med 2002;347:305–13.
5. Haass M, Kitzman DW, Anand IS, et al. Body mass index and adverse cardiovascular outcomes in heart failure patients with preserved ejection fraction: results from the irbesartan in heart failure with preserved ejection fraction (I-PRESERVE) trial. Circ Heart Fail 2011;4:324–31.
6. Kindel TL, Strande JL. Bariatric surgery as a treatment for heart failure: review of the literature and potential mechanisms. Surg Obes Relat Dis 2018;14:117–22.
7. Ashrafian H, le Roux CW, Darzi A, Athanasiou T. Effects of bariatric surgery on cardiovascular function. Circulation 2008;118:2091–102.
8. Aggarwal R, Harling L, Efthimiou E, et al. The effects of bariatric surgery on cardiac structure and function: a systematic review of cardiac imaging outcomes. Obes Surg 2016;26:1030–40.
9. Alexander JK, Peterson KL. Cardiovascular effects of weight reduction. Circulation 1972;45:310–8.
10. Haufe S, Utz W, Engeli S, et al. Left ventricular mass and function with reduced-fat or reduced-carbohydrate hypocaloric diets in overweight and obese subjects. Hypertension 2012;59:70–5.
11. De las Fuentes, Waggoner AD, Mohammed BS, et al. Effect of moderate diet-induced weight loss and weight regain on cardiovascular structure and function. J Am Coll Cardiol 2009;54:2376–81.
12. Kindel TL, Foster T, Goldspink P, et al. Early weight loss independent effects of sleeve gastrectomy on diet-induced cardiac dysfunction in obese, Wistar rats. Obes Surg 2017;27:2370–7.
13. Medhora M, Gao F, Glisch C, et al. Whole-thorax irradiation induces hypoxic respiratory failure, pleural effusions and cardiac remodeling. J Radiat Res 2015;56:248–60.
14. Vest AR, Patel P, Schauer PR, et al. Clinical and echocardiographic outcomes after bariatric surgery in obese patients with left ventricular systolic dysfunction. Circ Heart Fail 2016;9:e002260.
15. Alpert MA, Lambert CR, Panayiotou H, et al. Relation of duration of morbid obesity to left ventricular mass, systolic function and diastolic filling, and effect of weight loss. Am J Cardiol 1995;76:1194–7.
16. Alpert MA, Terry BE, Lambert CR, et al. Factors influencing left ventricular systolic function in nonhypertensive morbidly obese patients, and effect of weight loss induced by gastroplasty. Am J Cardiol 1993;71:733–7.
17. Karason K, Wallentin I, Larsson B, Sjostrom L. Effects of obesity and weight loss on left ventricular mass and relative wall thickness: survey and intervention study. BMJ 1997;315:912–6.
18. Cuspidi C, Rescaldani M, Tadic M, et al. Effects of bariatric surgery on cardiac structure and function: a systematic review and meta-analysis. Am J Hypertension 2014;27:146–56.
19. Mikhalkova D, Holman SR, Jiang H, et al. Bariatric Surgery-induced cardiac and lipidomic changes in obesity-related heart failure with preserved ejection fraction. Obesity 2018;26:284–90.
20. Sorop O, Heinonen I, van Kranenburg M, et al. Multiple common comorbidities produce left ventricular diastolic dysfunction associated with coronary microvascular dysfunction, oxidative stress, and myocardial stiffening. Cardiovasc Res 2018;114:954–64.
21. Varga ZV, Kupai K, Szucs G, et al. MicroRNA-25-dependent up-regulation of NADPH oxidase 4 (NOX4) mediates hypercholesterolemia-induced oxidative/nitrative stress and subsequent dysfunction in the heart. J Mol Cell Cardiol 2013;62:111–21.
22. Csont T, Bereczki E, Bencsik P, et al. Hypercholesterolemia increase myocardial oxidative and nitrosative stress thereby leading to cardiac dysfunction in apoB-100 transgenic mice. Cardiovasc Res 2007;76:100–9.
23. Zucker LM, Zucker TF. Fatty, a new mutation in the rats. J Heredity 1961;52:275–8.
24. Bray GA, York DA. Studies on food intake of genetically obese rats. Am J Physiol 1972;223:176–9.