Dependence of Cardiac Systolic Function on Elevated Fatty Acid Availability in Obese, Insulin-Resistant Rats.

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Highlights

- *Ex vivo* cardiac pump function of obese rats is compromised when glucose is the only substrate
- Fatty acids normalise cardiac pump function *ex vivo* in hearts from obese rats
- Elevated fatty acid levels are important to maintain normal LV systolic function in obesity

Abbreviations

FA, fatty acid

LV, left ventricular

LV Ees, the slope of the LV systolic pressure-volume relationship

LV En, left ventricular end systolic elastance

LVEDD, Left ventricular end diastolic diameter

LVESD; left ventricular end systolic diameter

FSend; endocardial fractional shortening

FSmid, midwall fractional shortening

ABSTRACT

Background: Clinical data advocating an adverse effect of obesity on left ventricular (LV) systolic function independent of comorbidities is controversial. We hypothesized that in obesity with pre-diabetic insulin resistance, circulating fatty acids (FA's) become a valuable fuel source in the maintenance of normal systolic function.

Methods: Male Wistar rats were fed a high caloric diet for 32 weeks to induce obesity. Myocardial LV systolic function was assessed using echocardiography and isolated heart preparations.

Results: Aortic output was reduced in obese rat hearts over a range of filling pressures (for example: 15 cmH₂O, obese: 32.6±1.2 ml/min vs. control: 46.2±0.9 ml/min, p<0.05) when perfused with glucose alone. Similarly, the slope of the LV end-systolic pressure-volume relationship decreased, and there was a right shift in the LV end-systolic stress-strain relationship as determined in Langendorff perfused, isovolumic rat heart preparations in the presence of isoproterenol (10⁻⁸M) (LV En, obese: 791±62 g/cm² vs. control: 1186±74 g/cm², p<0.01). The addition of insulin to the perfusion buffer improved aortic output while the addition of FA's completely normalized aortic output. LV function was maintained in obese animals *in vivo* during an inotropic challenge.

Conclusions: Elevated circulating FA levels may be important to maintain myocardial systolic function in the initial stages of obesity and insulin resistance.

Key words: obesity, fatty acids, cardiac systolic function.

INTRODUCTION

Obesity is an independent risk factor for heart failure.¹ However, whether obesity promotes cardiac pump dysfunction through mechanisms unrelated to hypertension and diabetes is still uncertain. In this regard, clinical studies conducted with overweight to severely obese patients have demonstrated either augmented^{2,3} preserved⁴⁻⁸ or reduced⁹ left ventricular (LV) systolic function independent of diabetes and hypertension. Other authors additionally report reduced load independent measures of systolic function (subclinical systolic dysfunction) with preserved ejection fraction in similar participants.^{10,11} Data from animal studies also provide inconclusive observations regarding the effect of diet induced obesity on LV systolic function.¹²⁻¹⁹

One potential explanation for the conflicting data regarding the independent effect of obesity on cardiac systolic function is that obesity may indeed be associated with intrinsic myocardial alterations that impair contraction, but that at least in the early stages of obesity, compensatory myocardial changes may occur that enable myocardial function to be preserved. This is supported by the observation that the duration of especially morbid obesity is associated with a reduction in LV systolic function (LV fractional shortening).²⁰ In line with this hypothesis, obesity is associated with increased circulating fatty acid (FA) concentrations²¹ and in most instances elevated rates of myocardial FA oxidation.^{16,22} Since the myocardium relies less on glucose as a fuel source in the setting of obesity and insulin resistance,^{16,22} it is plausible that increased FA availability combined with enhanced FA oxidation becomes vital to help maintain myocardial mechanical function in hearts that are metabolically compromised. We therefore tested

the hypothesis that elevated circulating FA's and insulin associated with obesity and insulin resistance may compensate for an impaired myocardial systolic function noted in obese, insulin-resistant (pre-diabetic) rats when glucose is present as the only fuel source. In order to test this hypothesis we studied cardiac mechanical function in a previously described animal model of obesity and insulin-resistance.^{12,18,23,24} In the present study cardiac systolic function was determined both *in vivo* and *ex vivo*. In the *ex vivo* studies, we assessed cardiac mechanical performance in the absence and in the presence of FA's and insulin at concentrations found *in vivo* in lean and obese rats.

METHODS

Animals

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). Sixty male Wistar rats weighing 200 ± 10 grams received a standard rodent chow supplemented with sucrose and condensed milk for 32 weeks. The experimental diet is designed to induce obesity through hyperphagia with the experimental group ($n=31$) consuming 570 ± 23 kJ/day as compared to the control group ($n=29$) that consumed 371 ± 18 kJ/day.^{12,25} The compositions of the standard rat chow and the experimental diet used in this study have previously been reported.²⁴ We have previously demonstrated that this experimental diet induces both obesity and insulin resistance after 16 weeks of consumption.^{23,26} Blood pressures were determined using a tail cuff method.²⁷

Isolated, perfused heart preparations.

To assess whether the absence of circulating FA's and insulin resulted in cardiac pump dysfunction, left ventricular systolic function was assessed ex vivo in 1) the isolated working heart model (as modified by Opie et al²⁸) to ensure that systolic function was assessed in a heart preparation performing work and 2) in the isolated retrograde perfused, isovolumic, constant coronary flow heart preparations²⁹ to account for the effects of load on systolic function; to assess intrinsic myocardial systolic function (see below); and to account for potential effects of obesity on coronary flow.

Rats were anesthetized with an intraperitoneal injection of pentobarbitone-sodium (60 mg/kg) and the hearts rapidly excised. The hearts were placed in ice-cold buffer before being transferred to a heart perfusion apparatus where they were perfused with a Krebs-Henseleit buffer equilibrated with 95%O₂ and 5%CO₂ at 37°C (pH 7.4) (in mmol/L- NaCl 118.0, KCl 4.7, MgSO₄.7H₂O 1.2, CaCl₂ 1.25, NaHCO₃ 25, KH₂PO₄ 1.2, glucose 10 (Merck Pty.Ltd., Darmstadt, Germany)) at a pressure of 100cm H₂O. Retrograde perfusion was initiated within 45 seconds of excision of the heart.

The influence of FA's or insulin (or the combination of both) on LV systolic function was only assessed in working heart preparations. FA's (final concentration: 0.7mM for control and 1.5mM for obese rats) were pre-bound to 3% bovine serum albumin (Fraction V, Roche, Germany) and added to the perfusion buffer as previously described.³⁰ FA's in the bovine serum albumin contributed 0.3 mM to the FA concentration in the buffer and the rest of the FA's were added in the form of palmitate (Sigma-Aldrich, St.Louis, MO). Insulin (humulin-N, Eli Lilly, South Africa) was added to the perfusion buffer to achieve concentrations of 30μIU/ml for control hearts and 50μIU/ml in obese rat hearts. These

FA and insulin concentrations closely resemble the plasma concentrations we previously reported in control and obese rats following 16 weeks of feeding.²³

Working heart model: For working heart perfusions the left atrium was cannulated and hearts were perfused in the working heart mode initially at a preload of 15 cmH₂O. The preload was subsequently increased at five minute intervals from 15 to 17.5 cmH₂O and then to 20 cmH₂O. Afterload was maintained at 100 cmH₂O. Hearts perfused with glucose or glucose and insulin as the only substrate were paced at 330 bpm with the stimulation voltage 10% above threshold via platinum wire electrodes attached to the left atrium and the apex of the heart. Hearts perfused in the presence of glucose and FA's were not paced. The heart rates from these un-paced hearts were however comparable between control and obese animals (see results section). Hearts with a rate below 200 bpm were excluded from the study (n=1). During FA and insulin perfusion, the perfusion buffer (200ml) was recirculated for the duration of the experiment. This was not the case when glucose was used as the sole substrate. Coronary flow and aortic output were documented. Systolic performance was assessed from aortic output determined at incremental filling pressures.

Isovolumic, retrograde perfusion model: These studies were performed in a separate group of rats from those used for the working heart preparations, using an approach previously described.³¹ Once hearts were mounted on the perfusion apparatus, the coronary flow rate was determined volumetrically and adjusted to achieve a constant flow of 12 ml/min/g heart weight. The hearts were paced at 300 bpm as described above. LV developed pressure was determined by use of a water-filled balloon-tipped cannula coupled to a pressure transducer inserted via the left atrium into the LV cavity. A thin-

walled latex balloon with a zero pressure filling volume beyond maximum LV lumen capacities was selected for this study to avoid the stiffness of the balloon wall contributing to LV pressure at higher filling volumes. The volume of the balloon wall was assessed with a water-displacement technique, and the same balloon was used throughout each of the studies. A micromanipulator was used to gradually increase LV volumes to values that resulted in no further change in LV developed pressure. LV pressures were determined at as many multiple small increments in volume as were practically possible to improve the accuracy of curve fitting during later analysis. A range of LV pressures and volumes were determined with (-)-isoproterenol HCl (Sigma-Aldrich, South Africa) added to the perfusion buffer (10^{-8} M). This approach was employed to assess peak systolic function. Load-independent LV systolic chamber performance was determined from comparisons of the slope of the LV systolic pressure-volume relationship (LV Ees) and load-independent intrinsic systolic myocardial function from the slopes of the LV stress-strain relations (systolic myocardial elastance, E_n).³²

Echocardiography

Left ventricular systolic function was determined in vivo using two-dimensional targeted M-mode echocardiography performed using a 7.5 MHz transducer and a Hewlett Packard Sonos 2500 sector scanner according to the American Society of Echocardiography convention as previously described.³¹ Rats were anesthetized with ketamine and xylazine³¹ and echocardiography was performed blinded, and in random order by a single observer (GRN). Two scans were obtained from each rat and the mean values were calculated. LVEDD and LVESD and posterior wall thickness were measured using the leading edge technique.³¹ These measurements were obtained at baseline and then again

at regular intervals for 45 minutes after having injected 0.01 mg/kg (-)-isoproterenol HCl into the peritoneal cavity. This approach was employed to assess systolic functional reserve. Left ventricular systolic chamber function was determined from LV endocardial fractional shortening (LV FSend) and systolic myocardial function from LV midwall fractional shortening (LV FSmid) calculated as previously described.³¹

Blood analysis

To determine the impact of the model of obesity on circulating insulin, blood glucose, lipids, epinephrine and norepinephrine concentrations and percentage glycosylated haemoglobin (HbA1c), blood samples were obtained from the thoracic cavity immediately after extirpation of the heart. Blood glucose and lipids were determined with a glucometer (Gluco Plus™; distributed by Cipla Dibcare, Bellville, South Africa) or CardioCheck lipid analyser (CardioCheck™, Polymer Technology Systems Inc, Indianapolis, USA). Whole blood collected in EDTA tubes was used to determine HbA1c using a Siemens ADVIA 1800, according to the instructions of the manufacturers. Blood samples were collected in serum separation tubes, and centrifuged at 3000rpm at 4°C within 30 minutes of collection. Serum insulin concentrations were determined using a commercially available radioimmunoassay (Coat-A-Count® Insulin, Diagnostic Products Corporation, LA, USA). Epinephrine and norepinephrine concentrations were determined using a commercially available radioimmunoassay (IBL KatCombi, Hamburg, Germany).

Myocardial glycolytic flux rate determinations

Myocardial glycolytic flux rates were measured in hearts from control and obese rats. To determine the rate of myocardial glycolytic flux, the amount of ³H₂O released from the metabolism of 5-[³H]glucose by triosephosphate isomerase and enolase steps in the

glycolytic pathway was measured. Hearts were perfused with 5- ^3H glucose under normoxic conditions and coronary effluent samples collected for glycolytic flux rate determinations as described previously.²³

Statistical analysisAnalyses were performed using GraphPad Prism 5.1 Software.

All data are presented as mean $\pm$ standard error of the mean. When comparisons between two groups (diet and control groups) were made, an unpaired Students t-test was performed. Comparisons of the aortic output between the diet and control groups at three different preloads were made using a repeated measures ANOVA followed by the Tukey *post hoc* test. To compare load independent systolic chamber function between the diet and control groups, the mean slope values in each group were derived from the slopes of linear regressions of the linear portion of the LV systolic pressure-volume curve (end systolic elastance, LV Ees) in individual rats. Comparisons of the mean slope values between the diet and control groups were made using a Students t-test. Comparisons of the aortic output between the diet and control groups with and without FA's at three different preloads were made with a repeated measures ANOVA followed by the Tukey *post hoc* test. To assess the effects of exposure of the myocardium to an inotropic stimulus on systolic function in the diet and control groups, a repeated measures ANOVA was performed followed by the Tukey *post hoc* test.

RESULTS

Characteristics of the obesity model

Table 1 shows the impact of the experimental diet on measures of adiposity, ventricular weight, blood biochemistry and systolic blood pressure. Rats fed the experimental diet were approximately 22% heavier, but had a 100% larger visceral fat mass than control diet fed rats. The obese rats further had a greater LV weight as compared to control rats. Although fasting blood glucose concentrations and HbA1c levels were similar between the two groups, insulin concentrations tended to be elevated while triglyceride levels were doubled in the obese rats when compared to the control rats. Circulating norepinephrine concentrations were elevated in the obese as compared to the control rats, but tail cuff systolic blood pressures were unchanged.

Myocardial glycolytic flux rates in the presence and absence of insulin

Myocardial glycolytic flux rates were reduced in obese hearts perfused with glucose alone (Control vs. Obese: no insulin; $0.56\text{nmol/g/min} \pm 0.07\text{nmol/g/min}$ vs. $0.30\text{nmol/g/min} \pm 0.01\text{nmol/g/min}$, $n=6-7$, $p<0.05$) and was increased by *in vivo* insulin concentrations in both groups of hearts (Control vs. Obese: plus insulin; $2.20\text{nmol/g/min} \pm 0.17\text{nmol/g/min}$ vs. $3.68\text{nmol/g/min} \pm 0.28\text{nmol/g/min}$, $n=6-7$, $p<0.05$). The insulin induced normalization of the glucose flux rate seen in the obese, insulin resistant hearts was not associated with a matched normalization of the aortic output in these hearts.

Effects of obesity on ex vivo systolic chamber function as assessed in the absence of fatty acids or insulin

Figure 1 shows the impact of obesity on systolic chamber function as determined using working heart preparations (Figure 1A) and in retrograde perfused, isovolumic, constant

coronary flow preparations (Figure 1B) in the absence of FA's and insulin. Irrespective of cardiac preloads, hearts from obese rats generated a reduced aortic output compared to that observed in the control rats (Figure 1A). Consistent with data obtained in the working heart preparation, in retrograde perfused, isovolumic, constant coronary flow preparations, hearts from obese rats had a right shift in the LV systolic pressure-volume relationship and a reduced LV Ees (Figure 1B) as compared to that observed in the control rats.

Effects of obesity on ex vivo intrinsic systolic myocardial function in the absence of fatty acids and insulin

The impact of obesity on intrinsic systolic myocardial function as determined in retrograde perfused isovolumic, constant coronary flow heart preparations is demonstrated in Figure 2. Hearts from obese rats displayed a right shift in the LV systolic stress-strain relationship and a reduced load-independent intrinsic systolic myocardial function (LV En) (Figure 2) as compared to that observed in the control rats. Thus, the impact of obesity on systolic chamber function (Figure 1) was in-part attributed to detrimental changes in intrinsic myocardial function.

Effects of exposure of the myocardium to fatty acids or insulin or the combination of both on ex vivo systolic cardiac function

The effect of relevant in vivo concentrations of FA's on systolic chamber function can be seen in Figure 3. FA exposure produced a marked inotropic effect in obese rat hearts, but had no effect on control rat hearts. Consequently, in the presence of in vivo concentrations of FA's, the reduced aortic output previously observed in the obese group when perfused with glucose alone (Figure 1A), was normalized as compared to control

rat hearts. In these un-paced perfusions, heart rates were comparable between the control and obese groups at all preloads (Control vs. Obese: 15cmH₂O: 279±11 bpm vs. 249±12 bpm, p=0.10; 17.5cmH₂O: 284±11 bpm vs. 267±10 bpm, p=0.27; 20cmH₂O: 286±12 bpm vs. 265±9 bpm, p=0.21).

The addition of relevant *in vivo* concentrations of insulin improved aortic output in obese but not control hearts (Figure 3B). These improvements in obese hearts were however small. Aortic outputs in obese, insulin perfused hearts were still lower than for corresponding control, insulin perfused hearts. The combination of *in vivo* concentrations of FA's with the insulin had no effect on aortic output in either the control or obese rat hearts when compared with FA's alone. With FA's and insulin control and obese hearts had similar aortic outputs at all preloads (Control vs. Obese: 15cmH₂O: 44.2±1.2 ml/min vs. 46.2±1.8 ml/min; 17.5cmH₂O: 42.9±1.3 ml/min vs. 46.2±1.8 ml/min; 20cmH₂O: 48.8±1.4 ml/min vs. 48.8±1.5 ml/min, n=7).

In vivo LV dimensions and systolic chamber and myocardial function

Table 2 and Figure 4 show the impact of obesity on LV dimensions and systolic chamber (FSend) and myocardial (FSmid) function as determined *in vivo* using echocardiography. Figure 4 additionally shows the impact of obesity on FSend and FSmid after isoproterenol injection. Obese rat hearts had comparable LVEDD and LVESD, but increased LV posterior wall thickness in comparison to control rats (Table 2). Irrespective of whether systolic function was assessed in the absence (Table 2) or in the presence of a marked inotropic stimulus (Figure 4), hearts from obese rats had a similar isoproterenol-induced increase in systolic function as compared to control rats when exposed to endogenous circulating FA's.

DISCUSSION

The main findings of the present study are as follows: In an animal model of obesity and insulin resistance, yet with comparable blood pressure and glycemic indices relative to the control group, we unmasked an inability to maintain LV mechanical function *ex vivo* when glucose was provided as the sole fuel source. This was evident from reduced LV systolic chamber (reduced aortic outputs over a range of comparable preloads and a reduced LV Ees and LV En) function in the absence of elevated circulating FA and insulin concentrations (but similar glucose concentrations). However, exposure of these hearts to FA concentrations representative of *in vivo* concentrations of FA's resulted in increased aortic outputs (normalized function relative to controls) as determined over a range of preloads in the obese group. The ability of elevated levels of FA's to normalize systolic function *ex vivo* in obese rat hearts, may in-part explain the ability of obese rats to generate normal chamber (FS_{end}) and myocardial (FS_{mid}) systolic function *in vivo* at baseline and in the presence of a marked inotropic stimulus.

The healthy heart is thought to be metabolically flexible, in that it can effectively utilize various substrates in order to maintain LV function. This has been shown to be true when perfusing isolated hearts from healthy animals with either glucose alone, or in combination with FA in the present study and those of others.³³ However, the hearts ability to effectively use glucose as an energy source is compromised in obese insulin resistant states,³⁴ which, as we demonstrate, translates into reduced intrinsic systolic myocardial function and LV chamber function when the heart is forced to rely solely on

glucose as a fuel source. This observation is in line with a number of studies reporting reduced contractile function in isolated cardiomyocytes or *ex vivo* perfused hearts obtained from obese animals where glucose was provided as the sole substrate.^{12,18,35-37}

Our data show that although the addition of insulin improves cardiac function in our obese insulin resistant animals, the mechanical function is still significantly compromised when compared to control animals. We however demonstrate that with the inclusion of *in vivo* representative levels of FA's to the perfusate, hearts from obese rats developed an increased pump function to levels commensurate with control values at all the preloads assessed. It is unlikely that augmented loading conditions may account for these observations in the working heart perfusion model since preload, afterload and heart rate were comparable between the FA perfused hearts from the control and obese animals. This observation may therefore in part explain the reduced contractile function noted in the previously mentioned studies,^{12,18,35-37} as well as the comparable myocardial function noted in obese insulin resistant rats relative to controls *in vivo* during resting conditions and following inotropic stimulation (present study). Surprisingly, the combined inclusion of both insulin and FA's in the perfusion buffer in obese hearts did not have an additive effect. Systolic function (aortic output) remaining unchanged when compared to the hearts treated with FA's only. The myocardial dependence on elevated levels of FA's as a fuel to maintain LV systolic function in obese rats is in keeping with the preserved *in vivo* LV systolic function noted in other diet induced models of obesity.^{13,19} Our results thus emphasize the importance of including FA as a fuel source when assessing *ex vivo* contractile parameters in models of obesity and insulin resistance.

Interestingly, the normal LV pump function noted in obese mice relative to controls in the study of Yan et al¹⁷ occurred in the absence of elevated circulating FA levels, a finding that differs from the model used in the present study where circulating FA's were previously reported to be elevated.²³ Thus it is possible that in obesity and insulin resistance, the presence of FA's per se and possibly the heart's ability to use this fuel source effectively may be equally important in the maintenance of LV pump function. We therefore speculate that during the myocardial metabolic adaptation phase of obesity, prior to the development of frank diabetes and hypertension, energy derived from FA metabolism plays a much more important role in maintaining LV pump function and functional reserve than in healthy hearts from lean controls.

Although we did not investigate substrate metabolism or identify the expression of genes involved in glucose and fatty acid metabolism in detail, it is clear from our glycolytic flux rate determinations that in the very early stages of diet induced obesity, cardiomyocyte glycolysis and glucose oxidation rates are decreased. This is possibly due to a reduction in GLUT4 expression and attenuated insulin stimulated GLUT4 translocation which may lead to increased FA oxidation and an increase in myocardial oxygen consumption.³⁸ These changes occur prior to increased expression of peroxisome proliferator activated receptor-alpha target genes.³⁸ It is reasonable to believe that this change in substrate flexibility is inevitably aimed at maintaining adequate myocardial energy production to preserve LV pump function although it may come at the expense of reduced cardiac efficiency. Importantly, these changes have been shown to occur in the obese insulin

resistant animals prior to the development of diabetes associated systolic dysfunction.³⁴ Despite the important role of elevated FA's on myocardial function in obesity it is further clear that a persistent increase in circulating FA levels is associated with adverse myocardial consequences such as atrial fibrillation³⁹ and diastolic dysfunction⁴⁰ whereas increased myocardial FA uptake and the consequent storage thereof within heart tissue are associated with reduced LV systolic function in humans with impaired glucose tolerance.⁴¹ In line with this observation, Peterson et al⁴² has also speculated that the altered myocardial substrate utilization profile associated with obesity and insulin resistance may contribute to cardiac dysfunction. It may therefore be speculated that detrimental consequences of increased FA supply to the myocardium are only seen when increased FA supply exceeds the hearts ability to oxidize the FA⁴³ which in turn may lead to the formation of toxic lipid intermediates.^{44,45} Alternatively, long term exposure of the heart to high levels of a particular substrate may render the metabolic network of the heart incapable of adapting to a superimposing metabolic stress, consequently resulting in cardiac dysfunction.¹⁷ Although it may seem reasonable to attempt to modify myocardial substrate metabolism by inhibiting FA oxidation and increasing glucose oxidation, Yan et al¹⁷ demonstrated that this could also be disadvantageous to the heart in the setting of obesity, therefore suggesting that this approach should be pursued with caution. This notion is supported by the present study as the importance of FA's as a fuel source for the heart in the obese insulin resistant condition is clearly demonstrated. The complicated balance between FA availability and cardiac function was further demonstrated by various authors where an acute to short term reduction in circulating FA levels

significantly reduced cardiac function in patients with heart failure and healthy participants respectively.^{46,47}

The increased systolic functional response to FA's in obese, but not control rat hearts may not be the only explanation for the preserved pump function noted *in vivo* in the present study. Elevated circulating norepinephrine concentrations have been noted in obese humans⁴⁸ and also in obese rats in the present study, which may enhance pump function *in vivo*. These elevated norepinephrine levels may not only have direct inotropic effects but potentially also contribute to increased lipolysis,⁴⁹ fatty acid availability and β -oxidation⁵⁰ which potentially also contributes to the significant improvement in systolic function seen with the inclusion of FA's.

Although myocardial contractile dysfunction may be due to both diastolic and systolic dysfunction, we did not assess diastolic function in the hearts in this study. This aspect of cardiac dysfunction more difficult to assess and fell beyond the scope of this study. While we did not report data documenting *in vivo* free FA levels in this study, we have previously demonstrated that rats fed this high caloric diet for 16 weeks have circulating free FA levels that are double that of the control group.²³ This study importantly used relevant *in vivo* concentrations of FA for control and obese animals when assessing their impact on cardiac pump function. In addition comparisons between *in vivo* and *ex vivo* functional parameters utilized in this study provide a more comprehensive assessment of cardiac systolic function in these hearts.

In conclusion, the present study indicates that cardiac systolic dysfunction occurs in obesity with insulin-resistance in the absence of FA and insulin. However in the presence of normally elevated *in vivo* concentrations of FA's, cardiac systolic function is preserved in the isolated heart thus mimicking *in vivo* myocardial pump function. The study thus demonstrates the importance of free FA's in the maintenance of cardiac systolic function at rest and in response to inotropic stimulation in obesity and insulin resistance.

Disclosure

No conflicts of interest are declared by the authors. All authors have approved the final article.

Author Contributions

Writing the paper and scientific input towards the paper: WS, EFdT, GRN, AJW, AL;

Experimental work: EFdT, GRN, WS, AJW; Statistical analysis: WS, EFdT, AJW.

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Legends.

Figure 1. Impact of obesity on ex vivo left ventricular (LV) systolic chamber function in the absence of exposure of the myocardium to fatty acids in the coronary perfusate. Panel A illustrates aortic outputs at incremental filling pressures in working heart preparations, and panel B shows LV systolic pressures over a range of filling volumes in isovolumic retrograde perfused hearts. Comparison of the slopes of these relationships (LV Ees) are shown in the insert to panel B.

* $p < 0.05$ versus controls.

Figure 2. Impact of obesity on ex vivo left ventricular (LV) intrinsic myocardial systolic function, as assessed from LV systolic stress-strain relations in isovolumic heart preparations in the absence of exposure of the hearts to fatty acids in the coronary perfusate. Comparisons of the slopes of these relations (LV En) are shown in the insert.

* $p < 0.05$ versus controls.

Figure 3. A. Impact of obesity on ex vivo left ventricular (LV) systolic chamber function in the absence as compared to the presence of fatty acids in the coronary perfusate.

* $p < 0.05$ versus controls and diet +FA. B. Impact of obesity on ex vivo left ventricular (LV) systolic chamber function in the absence as compared to the presence of insulin in the coronary perfusate.* $p < 0.05$ versus controls. † $p < 0.05$ vs. control + insulin

Figure 4. Impact of obesity on left ventricular systolic chamber (endocardial fractional shortening [FS]) (panel A) and myocardial (midwall FS) (panel B) function at baseline and after an intraperitoneal injection of (-)-isoproterenol HCl (0.01 mg/kg).

* $p < 0.001$ versus baseline.