Lowering Body Weight in Obese Mice with Diastolic Heart Failure Improves Cardiac Insulin Sensitivity and Function: Implications for the Obesity Paradox

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Abstract:

Recent studies suggest improved outcomes and survival in obese heart failure patients (i.e. the obesity paradox), although obesity and heart failure unfavourably alters cardiac function and metabolism. We investigated the effects of weight loss on cardiac function and metabolism in obese heart failure mice. Obesity and heart failure were induced by feeding mice a high fat diet (HF, 60% kcal from fat) for 4 wk, following which an abdominal aortic constriction (AAC) was produced. Four wk post-AAC, mice were switched to a low fat diet (12% kcal from fat; HF AAC LF) or maintained on a HF (HF AAC HF) for a further 10 wk. At 18 wk, HF AAC LF mice weighed less than HF AAC HF. Diastolic function was improved in HF AAC LF mice, while cardiac hypertrophy was decreased, and accompanied by decreased SIRT1 expression, increased FOXO1 acetylation, and increased atrogin-1 expression compared to HF AAC HF mice. Insulin stimulated glucose oxidation was increased in hearts from HF AAC LF, compared to HF AAC HF. Thus, lowering body weight by switching to LF diet in obese mice with heart failure is associated with decreased cardiac hypertrophy, and improvements in both cardiac insulin sensitivity and diastolic function, suggesting that weight loss does not negatively impact heart function in the setting of obesity.

Key words: Diastolic dysfunction, insulin resistance, glucose oxidation, fatty acid oxidation, obesity paradox, cardiac hypertrophy
Introduction:

Obesity is a recognized risk factor for heart failure (1,2). Obesity is associated with left ventricular hypertrophy and dilatation, features that are known to precede the development of overt heart failure (3,4). For every increase in body mass index (BMI) by 1 the risk of heart failure increases by 5% in men and 7% in women (5). In a prospective study of 21,094 men, every 1 kg/m² increase in BMI was associated with an 11% increase in heart failure risk (6). Compared with lean subjects, overweight and obese individuals have a 49% and 180% increased risk of developing heart failure, respectively.

Despite the fact that obesity increases the incidence of heart failure, several studies suggest that there is a protective effect of being obese in patients with heart failure, known as the “obesity paradox” (7-13). A low BMI in heart failure patients is associated with decreased survival. This paradoxical association is found in patients with preserved and reduced ejection fraction, with a nadir of mortality in one individual patient meta-analysis (n=23,967) of 34.0-34.9 kg/m² (8). A number of experimental studies have also shown favourable effects of high fat (HF) diet on cardiac function and survival in different disease states such as myocardial infarction, heart failure and hypertension (see 14 for review).

The obesity paradox would suggest that intentional weight loss in obese heart failure patients could have a detrimental effect on cardiac function. However, weight loss can decrease cardiac hypertrophy and improve LV systolic and diastolic filling in obese heart failure patients (15,16).

The scientific literature offers inconsistent results with regards to the association between obesity and heart failure. It is therefore important to better understand how obesity impacts
outcomes of heart failure patients. One major pathway that is altered in both obesity and heart failure is cardiac energy metabolism (17,18). Insulin resistance states such as obesity and diabetes are associated with dramatic changes in cardiac energy metabolism, which include an increase in fatty acid oxidation, and a decrease in glucose oxidation (17,19,20). Diet-induced obese mice (20) as well as ob/ob and db/db mice (19) that exhibit insulin resistance have increased cardiac fatty acid oxidation rates and decreased efficiency. Obese women with increased myocardial fatty acid uptake and oxidation show insulin resistance and lower cardiac efficiency (21). Thus, obesity and the associated insulin resistance adversely affect cardiac metabolism and function.

Heart failure even in the absence of risk factors such as obesity can also lead to dramatic alterations in cardiac energy metabolism (17,18). A decrease in energy production and or a decrease in energy efficiency can result in a state of “energetic deficit” in the heart (22-24). Using various experimental models of heart failure, we have shown that a decrease in insulin-stimulated glucose oxidation precedes heart failure and that stimulating glucose oxidation can improve both cardiac efficiency and function (24-27).

Since both obesity and heart failure can profoundly alter cardiac energy metabolism, we investigated what effect lowering body weight by switching from high fat to low fat diet in obese mice with heart failure has on cardiac function and energy metabolism. This was achieved by developing a model of obesity and heart failure in mice, which involved producing diastolic dysfunction in obese mice via an abdominal aortic constriction (AAC). We then examined what effect weight loss due to switch from high fat to low fat diet in the obese mice with heart failure had on diastolic function, cardiac hypertrophy, and cardiac energy metabolism.
Methods:

Animals: All procedures were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. The care of mice conformed to the guidelines of the Canadian Council on Animal Care.

Obesity heart failure model: Male C57Bl/6J mice (8 wk of age) were randomly assigned to be fed either a standard chow/low fat diet (LF, 12% kcal from fat) or a high fat diet (HF, 60% kcal from fat). Four weeks later, mice in both groups were anesthetized with 0.75% isoflurane and underwent sham or abdominal aortic constriction (AAC) surgical procedure (24). Briefly, a 2 cm medio-lateral incision extending from the level of the 13th rib was made on the ventral side of the left abdominal wall 1.5 cm lateral to the spine. The abdominal aorta was located at the level of the adrenal gland. A titanium vascular clip was applied to constrict the aorta. It was set for a 0.11 mm closure. Sham operated animals were subjected to an identical surgical procedure except that a clip was not applied to the aorta. The surgical incision was then closed and the animals were allowed to recover under constant supervision.

Treatment protocol: Mice in the LF and HF groups that were subjected to either the sham or ACC surgery were continued on their respective diet for a further 4 wk period. At 4 wk post-surgery (8 week after starting the LF or HF diet) mice were assessed for body weight, whole body glucose tolerance, and in vivo cardiac function (see below). Furthermore, the pressure gradient across the AAC in the HF AAC HF mice was similar to that of the sub-group of mice that was subsequently randomized to be fed the low fat diet (HF AAC LF) (36.5±3.7 vs.
38.1±4.6 mm Hg). Mice in the HF AAC group were then randomly divided to either continuing on a HF diet, or switching to a LF diet for a further 10 wk period. The HF sham and LF sham groups were also continued on their respective diets for a further 10 wk period. Body weight in all mice was monitored weekly.

**Oral glucose tolerance test:** Oral glucose tolerance tests were performed at 4, 8, 14 and 18 wk of the protocol. After an overnight fast for 16 h, a fasting blood glucose sample was obtained. Subsequently, mice were challenged with glucose (2g/kg body weight) orally. Blood glucose was measured at 15, 30, 60, 90 and 120 min after glucose administration using an Accu Check Aviva (Roche Diagnostics) glucometer.

**Magnetic Resonance Imaging (MRI):** Fat and lean mass composition was analyzed after 18 wk of the feeding protocol using EchoMRI QMNR 4-in-1 Whole Body Composition Analyzer, Echo Medical Systems (Houston, TX, USA).

**Echocardiography and Tissue Doppler Imaging:** Echocardiography was performed using a Visualsonic Vevo 770 high-resolution echocardiography imaging system equipped with a 30-MHz transducer (RMV-707B; VisualSonics, Toronto, Canada) (26). Echocardiographic analyses of in vivo cardiac function were carried out at baseline (4 wk), 6, 8, 14 and 18 wk of the treatment protocol. Mice were anesthetized with 0.75% isoflurane for the duration of the procedure. M-mode images were obtained for measurements of left ventricular (LV) wall thickness, LV end-diastolic diameter, and LV end-systolic diameter. LV ejection fraction (%EF) and fractional shortening (%FS) were calculated to assess systolic function. Tissue Doppler
imaging (TDI) was used to assess diastolic function, where a reduction in E'/A' and an elevation in E/E' were considered markers of elevated LV filling pressure and diastolic dysfunction. TDI was utilized to characterize the inferolateral region in the radial short axis at the base of the LV with the assessment of early (E) and late diastolic (A) myocardial velocities.

**Isolated Working Heart Perfusions:** After 18 wk of the treatment protocol, mice were euthanized with sodium pentobarbital. Hearts were quickly excised after a thoracic incision and cannulated, and perfused as isolated working preparations as described previously (24). Hearts were perfused with Krebs-Henseleit solution containing 2.5 mM Ca\(^{2+}\), 5 mM \([U^{-14}C]\)glucose, 0.8 mM \([9,10^{-3}H]\)palmitate pre-bound to 3% albumin. Hearts underwent an initial 30 min perfusion, at which time 100 µU/mL insulin was added to the perfusate, and hearts were perfused for an additional 30 min period. Glucose and palmitate oxidation rates were measured by simultaneously collecting \(^{14}\)CO\(_2\) and \(^3\)H\(_2\)O produced from the oxidation of \([U^{-14}C]\) glucose and \([9,10^{-3}H]\) palmitate, respectively, as described previously (24). At the end of the perfusion, hearts were quickly frozen with tongs cooled to the temperature of liquid N\(_2\).

**Determination of cardiac triacylglycerol:** Triacylglycerol from 10 mg of frozen heart tissue was extracted with a 2:1 chloroform-methanol solution and quantified with an enzymatic assay kit (Wako Pure Chemical Industries), as previously described (20).

**Short chain CoA determination:** Approximately 10 mg of frozen heart tissue was homogenized for 30 s using a Polytron Homogenizer in 200 µL of 6% (v/v) perchloric acid and 2 mM dithiothreitol. After homogenization the samples were left on ice for 10 min and then centrifuged
at 12,000 g for 5 min. The supernatant was collected and subjected to ultra performance liquid chromatography (UPLC) analysis, as described previously (28). For short chain CoA ester analysis, 10 ul of the sample was run through an Ascentis Express C18 column (10 cm x 2.1 mm and 2.7 um particle size, Supelco Oakville, ON, Canada). The flow rate was set at 0.4 ml/min and analyte detection occurred at an absorbance of 260 nm. The mobile phase consisted of a mixture of buffer A (250 mM NaH$_2$PO$_4$) and buffer B (250 mM NaH$_2$PO$_4$ and acetonitrile, pH 5.0). The gradient elution profile consisted of the following: 0–2.5 min, 97% A 3% B; 2.5–7.5 min, 97% A 3% B to 82% A 18% B; 7.5–15 min, 82% A 18% B; 15–18 min, 82% A 18% B to 63% A 37% B; 18–35 min, 63% A 37% B to 10% A 90% B; 35–42 min, 10% A 90% B. Peaks were integrated using the Beckman System Gold software package.

**Heart tissue preparation for immunoblot analysis:** Frozen ventricular tissue was homogenized for 30 s with a Polytron homogenizer in a homogenization buffer containing 0.05M Tris-HCl, 10% glycerol, 1mM EDTA, 0.02% Brij-35, and 1mM dithiothreitol in the presence of protease and phosphatase inhibitors (Sigma). Homogenized tissues were then centrifuged at 800 g for 10 min to obtain a supernatant lysate. Protein assay was performed using the Bradford method. Samples were boiled for 5 min in a sample preparation buffer containing (0.062M Tris-HCl, 10% glycerol, 0.003% bromphenol blue, 5% 2-β-mercapto-ethanol, 2% SDS, and 6M Urea).

**Immunoblot analysis:** Proteins (20 µg/lane) were subjected to 10% SDS-PAGE and transferred to nitrocellulose membranes (24). Membranes were blocked with 5% skim milk for 1 h and probed with specific antibodies against AMPK, P-AMPK, P-mTOR, mTOR, P-P70S6K, P70S6K, Akt, P-Akt, PDH, P-PDH, PDK4, GSK3β, P-GSK3β, SIRT1, FOXO1, Atrogin-1, P-38
MAPK, P-P38 MAPK (Cell Signaling Technology Inc., Danvers, MA), acetyl-lysine (Millipore Inc., Billerica, MA), SIRT3, LCAD (Abcam Inc., Toronto, ON). GCN5L1 was generously provided by Dr. M.N. Sack (NIH, Bethesda, MD). Membranes were incubated with the appropriate secondary antibodies (Santa Cruz Biotechnologies, Santa Cruz, CA) for 1 h. Enhanced chemiluminescence (BioRad Inc, Hercules, USA) system was used for band detection. The intensity of band signals was analyzed by Quantity One software (4.4.0).

**Immunoprecipitation:** 100 µg of the total heart lysate was used for immunoprecipitation. Lysates were incubated with acetyl-lysine antibodies (3µg/100µg lysate) overnight at 4°C. 50µl of protein A-agarose beads were added to each sample and incubated on a rotator for 4 h at 4°C. After 4 hours, samples were washed 3X with 100µl of homogenization buffer containing 3M NaCl and centrifuged at 16000g for 5 min. Immunoprecipitates were boiled in a sample preparation buffer for 5 min (25), and the resulting samples were subject to immunoblot analysis as described above.

**Statistical analysis:** Data are represented as means ± SEM. Data were analyzed by one-way or two-way analysis of variance (ANOVA). When ANOVA revealed differences, data sets were compared by Bonferroni's multiple-comparisons post-test. P<0.05 was deemed significant.
Results:

**Obesity heart failure model:** Following 4 wk of either LF or HF feeding, mice were subjected to a sham or AAC surgical procedure, and continued to receive either the LF or HF. At the 8 wk time point mice fed HF weighed significantly more than mice fed LF that were subjected to the sham surgical procedure (34.8±2.0 g, n=6 vs 26.1±1.2 g, n=6, P<0.05) (Fig. 1A). HF mice subjected to the AAC procedure had a similar body weight to HF sham. HF AAC mice were subsequently divided into a group that was switched to LF (HF AAC LF) or a group maintained on HF (HF AAC HF) for an additional 10 wk. At 18 wk the HF sham and HF AAC HF groups had significantly higher body weight compared to the LF sham group, while the HF AAC LF group did not gain weight, ultimately have weight similar to the LF sham group. Fat mass (g) assessed by MRI quantification was increased in both HF sham and HF AAC HF groups compared to LF sham, while the HF AAC LF group had decreased fat mass compared to HF sham and HF ACC HF groups (Fig. 1B). Lean mass (g) did not differ between the groups (Fig. 1C). Thus differences in body weight in these mice are primarily due to difference in fat mass. Impaired glucose tolerance had developed in HF mice at 8 wk as area under the curve (mmol/l·min) was significantly increased in both HF sham and HF AAC HF compared to the LF sham group (56.9±2.6, n=6 and 58.6±2.1, n=6, respectively, vs 46.1±1.2, n=6, P<0.05). At 18 wk glucose tolerance (mmol/l·min) was improved in HF AAC LF mice compared to both HF sham and HF AAC HF groups (44.5±1.4, n=5 vs 68.7±2.6, n=6 and 69.1±3.2, n=6, respectively, P<0.05).
Effects of weight loss due to diet switch from high fat to low fat on in vivo cardiac function in obese mice with heart failure: LF sham mice had a normal systolic and diastolic function while the HF sham mice developed diastolic dysfunction while maintaining a normal systolic function throughout the 18 wk study protocol (Suppl. Table 1). In HF mice subjected to an AAC, diastolic dysfunction occurred as early as 2 wk after the procedure (i.e. 6 wk into the protocol), and remained for the duration of the protocol as indicated by an increased E/A ratio (Fig. 2A). However, in mice switched to a LF diet at 8 wk (HF AAC LF) a significant improvement in diastolic function was seen (Fig. 2A, Suppl. Table 1). Mitral tissue doppler E'/A' ratio and E/E' ratio (marker of left atrial filling pressure) were also measured as indices of diastolic function. A significant decrease in the E'/A' ratio and an increase in the E/E' ratio were observed as early as 2 wk post-AAC in the HF AAC HF mice, demonstrating diastolic dysfunction. Diastolic dysfunction was attenuated in HF AAC LF mice, (Fig. 2B and Fig. 2C). Systolic function (%EF) was normal in LF sham and HF sham mice (Suppl. Table 1). AAC induced a significant reduction in % EF compared to baseline in HF mice (Fig. 2D). In the HF ACC LF mice a significant improvement in %EF was observed at the end of the 18 wk study period (Fig. 2D, Suppl. Table 1). Furthermore, as alterations in calcium handling can affect cardiac contractile function, we measured SERCA2a expression in these hearts. SERCA2a expression was significantly reduced in the HF AAC HF hearts and normalized in HF AAC LF hearts (Fig. 2E).

Mechanisms contributing to cardiac hypertrophy in obese mice with heart failure: The changes in cardiac function were accompanied by cardiac hypertrophy (Fig. 3A and Fig 3B) in the HF ACC HF groups. Cardiac hypertrophy was attenuated in the HF AAC LF groups. The heart weight / tibia length (HW/TL, mg/mm) ratio measured after 18 weeks also confirmed
cardiac hypertrophy in the HF AAC HF mice (Fig. 3B). The HW/TL in HF AAC HF (11.6±0.22, P<0.05) was significantly increased in comparison to LF Sham (9.1±0.33) and HF Sham (10.1±0.42) but was reduced in HF AAC LF (9.7±0.19). As such, weight loss due to diet switch from high fat to low fat resulted in a significant decrease in the severity of cardiac hypertrophy in these obese heart failure mice. To further characterize cardiac hypertrophy and failure, we also measured α-skeletal actin that is known to accumulate in hypertrophied hearts (29) and atrial natriuretic peptide (ANP) expression in these hearts. Both markers were significantly increased in the HF AAC HF hearts compared to LF Sham and reduced significantly in the HF AAC LF hearts (Fig 3C and Fig 3D).

Recent studies indicate that SIRT1 can regulate FOXO1, an important mediator of cardiac hypertrophy (30-34). SIRT1 expression was increased in HF AAC HF hearts (Fig. 3E), and accompanied by decreased FOXO1 acetylation (Fig. 3F), decreased atrogin-1 expression (Fig. 3G) and decreased p38 MAPK phosphorylation (Fig 3H). These changes were prevented in hearts from HF AAC LF mice. Interestingly, the activation of AMPK (phosphorylation) resulting in a phosphorylation and activation of p38 MAPK has also been suggested to increase atrogin-1 expression and decrease cardiac hypertrophy (35). Indeed, increased AMPK activation was observed in HF AAC LF hearts (Fig. 4A). In addition an increase in PKC-α (Fig. 4B) can also contribute to cardiac hypertrophy via phosphorylation and activation of mTOR (Fig. 4C) and subsequently P70S6K (Fig. 4D). Furthermore, a decrease in PKC-α and increased AMPK activation also result in decreased phosphorylation of mTOR and P70S6K resulting in decreased hypertrophy in the HF AAC LF mice. Accumulation of lipids such as triacylglycerol (TG) can also increase heart mass. HF AAC HF hearts had significantly higher TG content (6.3±0.4 µmol/g wet wt, P<0.05) in comparison to LF Sham (3.8±0.3 µmol/g wet wt) and HF Sham
(4.1±0.6 µmol/g wet wt) mouse hearts. In contrast, in the HF AAC LF mouse hearts no increase in TG levels (3.9±0.6 µmol/g wet wt) was observed compared to LF Sham mouse hearts.

**Cardiac energy metabolism in obese mice with heart failure:** Cardiac energy metabolism was assessed in isolated working hearts from each experimental group at the end of the 18 wk study. In hearts from the LF sham group, insulin increased glucose oxidation rates (Figure 5A), while inhibited fatty acid oxidation rates (Fig. 5B). This response was blunted in both HF Sham and HF AAC HF groups, indicating the presence of cardiac insulin resistance. Interestingly, in the HF AAC LF group a significant increase in insulin stimulated glucose oxidation was observed (Fig. 5A), which was accompanied by a significant insulin inhibition of fatty acid oxidation (Fig. 5B). In hearts from the HF AAC HF group, fatty acid oxidation dominated as source of ATP production (Fig. 5C) and TCA cycle acetyl CoA supply (Fig. 5D), even in the presence of insulin. In contrast, in the HF AAC LF group the contribution of glucose oxidation to ATP production and acetyl-CoA supply increased in the presence of insulin (Fig. 5 C and Fig. 5D).

While cardiac fatty acid oxidation rates can be regulated by malonyl CoA, glucose oxidation rates can be regulated by pyruvate dehydrogenase, the rate limiting enzyme in glucose oxidation. The content of malonyl CoA an endogenous potent inhibitor of mitochondrial fatty acid uptake was assessed in each group of hearts at the end of the 18 wk study protocol. Malonyl CoA levels were similar in LF sham, HF sham, and HF AAC HF mouse hearts (Suppl. Fig. 1A). HF AAC LF mouse hearts had significantly increased cardiac malonyl CoA content (Suppl. Fig. 1A). This was associated with a significant decrease in phosphorylation (inhibition) of acetyl CoA carboxylase, the enzyme responsible for malonyl CoA synthesis (Suppl. Fig. 1B). In contrast, the expression of malonyl CoA decarboxylase (which is responsible for malonyl CoA
degradation in) remained unchanged (Suppl. Fig. 1C). The increase in malonyl CoA content in the HF AAC LF group may contribute to the decrease in fatty acid oxidation, and increase in glucose oxidation observed in isolated working hearts in response to insulin treatment.

Interestingly, increased glucose oxidation rates in HF AAC LF mouse hearts was not accompanied by changes in PDH phosphorylation (which inhibits PDH activity) by pyruvate dehydrogenase (PDH) kinase 4 (PDK4) expression (Suppl. Fig 1D and 1E). On the other hand, a significant increase in the acetyl CoA/CoA ratio (which inhibits PDH activity) was found in HF Sham (0.13±0.02) and HF AAC HF (0.18±0.02) mouse hearts compared to LF sham hearts (0.10±0.01, P<0.05). Interestingly, cardiac acetyl CoA/CoA levels were normalized in HF AAC LF mouse hearts (0.10±0.01). These data demonstrate that hearts from obese mice with heart failure were insulin resistant, but that weight loss could improve insulin sensitivity.

**Effects of weight loss due to diet switch from high fat to low fat on the cardiac insulin signaling in obese mice with heart failure:** To examine the potential mechanisms responsible for the cardiac insulin resistance in obese mice with heart failure, we measured P-Akt levels, an important mediator of insulin sensitivity. The P-Akt/Akt ratio was lowest in the HF AAC HF mouse hearts, and also significantly increased in HF AAC LF mouse hearts (Fig. 6A). This correlated with a decrease in P-GSK3β in the HF AAC HF mouse hearts, and an increase in P-GSK3β in the HF AAC LF mouse hearts (Fig. 6B).

Furthermore, obesity and heart failure in HF Sham and HF AAC HF were associated with decreased GLUT4 expression in the membrane, while it was normalised in HF AAC LF hearts (Fig. 6C). However, no significant changes in membrane GLUT1 expression were observed among groups (Fig. 6D). To further understand mechanisms of insulin resistance, we also
measured the expression of SOCS3 (which is associated with insulin resistance) and its regulation by STAT3. Increased expression of SOCS3 was observed in HF Sham and HF AAC HF hearts, and was decreased in the HF AAC LF hearts (Fig. 6E). This was accompanied by parallel changes in phosphorylation (activation) of STAT3 (Fig. 6F).

**Effects of weight loss due to diet switch from high fat to low fat on acetylation control of fatty acid oxidation in obese mice with heart failure:** Recent studies have implicated post-translational changes in acetylation of fatty acid oxidation enzymes as an important pathway by which fatty acid oxidation is controlled (36-39). Important mediators include the mitochondrial acetyl-transferase GCN5L1, and the mitochondrial deacetylase SIRT3. Acetylation of lysine residues can occur on the fatty acid oxidative enzymes, long chain acyl CoA dehydrogenase (LCAD), which we have shown is associated with activation of LCAD activity and fatty acid oxidation rates (39). In HF sham and HF AAC HF mouse hearts, an increase in GCN5L1 expression was observed compared to LF sham hearts (Fig. 7A). The HF AAC LF hearts had significantly lower GCN5L1 expression compared to HF AAC HF hearts (Fig. 7A). However, changes in SIRT3 expression were not observed in any of the experimental groups (Fig. 7B). LCAD expression was significantly decreased in the HF AAC LF hearts compared to HF AAC HF mouse hearts (Fig. 7C). In parallel with the increase in GCN5L1 expression in the HF AAC HF mice, an increase in acetylation of LCAD was observed (Fig. 7D). This was accompanied by an increase in LCAD activity (Fig. 7E). In contrast, the decrease in GCN5L1 expression in HF AAC LF hearts (Fig. 7A) was associated with a decrease in LCAD acetylation (Fig. 7D), and a decrease in LCAD activity (Fig. 7E). We also found a positive correlation between abundance
of acetylated LCAD and palmitate oxidation rates suggesting that acetylation of LCAD could activate LCAD and therefore stimulate fatty acid oxidation in these hearts (Fig. 7F).

**Discussion:**

Our study provides a number of novel observations regarding the issue of diet induced weight loss in the setting of obesity and heart failure. First, weight loss significantly reduces the severity of existing heart failure in obese heart failure mice. This suggests that decreasing body weight does not aggravate heart failure (i.e. in contrast to the obesity paradox). We also demonstrate that weight loss or dietary changes from high fat to low fat diet in obese mice with heart failure has a number of desirable effects on heart metabolism and cardiac hypertrophy. In obese heart failure mice, the heart becomes insulin resistant and is almost completely reliant on fatty acid oxidation as a source of energy. Weight loss due to diet switch from high fat to low fat restored cardiac insulin sensitivity and switched energy substrate metabolism back towards glucose metabolism. Another desirable effect of weight loss is that cardiac hypertrophy was reduced in obese heart failure mice, which was due to an activation of anti-hypertrophic signalling pathways. Combined, our data clearly demonstrates that diet induced purposeful weight loss can dramatically lessen the severity of heart failure in obese mice.

As expected, obese heart failure mice exhibited a whole body insulin resistance. Of interest, is that the heart was also profoundly insulin-resistant in these obese heart failure mice. The ability of insulin to stimulate glucose oxidation and inhibit fatty acid oxidation was markedly impaired in the obese mice with heart failure. On the other hand, weight loss due to
switching these mice to a LF diet, resulted in a marked improvement in cardiac insulin sensitivity and a dramatic increase in glucose oxidation. This increase in cardiac insulin sensitivity correlates with improvements in the control of insulin signalling. STAT3 activation is associated with increased SOCS3 expression that is known to correlate with insulin resistance (40). SOCS3, in turn, inhibits insulin signaling by binding to the insulin receptor and inhibiting the tyrosine phosphorylation of IRS1 and IRS2 and the subsequent activation of Akt (41). The activation of STAT3 and increase in SOCS3 we observed in the hearts of obese heart failure mice is consistent with previous reports of increased SOCS3 expression in adipose tissue (42), liver (43,44) and skeletal muscle (45) that were associated with insulin resistance. This activation of STAT3 and increase in SOCS3 we observed in hearts of obese heart failure mice was associated with impaired insulin signaling (Akt and GSK3β phosphorylation) and therefore decreased glucose oxidation rates. Interestingly, a decrease in STAT3 activation (phosphorylation) and a decrease in SOCS3 expression was observed with weight loss in obese heart failure mice, resulting in improved insulin signaling, increased GLUT4 expression and increased glucose oxidation rates. However, it was interesting to note that although the HF Sham mice had a higher P-Akt/Akt ratio, the P-GSK3β was blunted in comparison to LF Sham hearts. GSK3β can be phosphorylated by PKC, PKA as well as p90RSK (46-48). The combined effect of lack of change in PKC and other kinases could have decreased GSK3β phosphorylation. Another factor that could potentially modify insulin signaling is leptin. While leptin is known to be increased in mice fed high fat diet in comparison to mice fed regular chow (49), whether alterations in insulin signaling and cardiac function were accompanied by changes in leptin levels is unclear in our study.
Despite the decrease in cardiac glucose oxidation rates in obese heart failure mice, no changes in PDK4 or p-PDH were observed. We therefore propose that the dramatic decrease in glucose oxidation in these hearts was therefore primarily occurring as a result of the observed increase in fatty acid oxidation, which competes with PDH for acetyl CoA production (i.e. the Randle Cycle)(50). As a result, the primary effect of impaired cardiac insulin signalling may have been on the fatty acid oxidative pathway. Insulin inhibition of cardiac fatty acid oxidation in obese heart failure mice was markedly impaired in obese heart failure mice. Weight loss due to switch to LF diet in these mice resulted in a dramatic improvement in the ability of insulin to inhibit fatty acid oxidation, and therefore increase glucose oxidation.

We recently showed that the decrease in cardiac function in mice subjected to pressure overload due to a transverse aortic constriction (27) or angiotensin II infusion (25,26) was associated with a decrease in insulin-stimulated cardiac glucose oxidation rates. We also showed that in mice lacking malonyl CoA decarboxylase, the enzyme that inhibits fatty acid oxidation through modulating malonyl CoA levels, a robust increase in cardiac insulin stimulated glucose oxidation was seen in obese mice (51). Therefore, we propose that the decrease in cardiac glucose oxidation and increase in fatty acid oxidation contributed to cardiac dysfunction in obese heart failure mice. However, while excessively high fatty acid oxidation rates may contribute to cardiac dysfunction, it should also be recognized that excessively low rates of fatty acid oxidation may also contribute to contractile dysfunction. For instance CPT-1B +/- mice that partially lack carnitine palmitoyltransferase-1 (CPT-1) have decreased cardiac fatty acid oxidation and develop cardiac dysfunction under mild pressure overload conditions (52). In addition, preserved cardiac function and attenuated cardiac hypertrophy are observed following pressure overload of mice in which fatty acid oxidation was increased by deletion of acetyl CoA
carboxylase (53). While maintaining adequate fatty acid oxidation may be important in heart failure, our data suggests that the excessively high rates of fatty acid oxidation in obese heart failure mice may also contribute to the severity of heart failure. In support of this concept, hearts from \textit{ob/ob} and \textit{db/db} mice have high fatty acid oxidation rates, low glucose oxidation rates and impaired cardiac function (19).

Recent studies have shown that mitochondrial acetylation of lysine residues is an important regulatory pathway involved in regulating fatty acid and glucose oxidation (36-39). Mitochondrial acetylation is a reversible process catalyzed by the acetyltransferase GCN5L1 (54,55), while deacetylation is mediated by sirtuins, particularly SIRT3 (56). We examined whether alterations in acetylation status contributed to the alterations in energy metabolism in the obese heart failure mice. Acetylation of the fatty acid oxidative enzyme LCAD was increased in obese mice with diastolic dysfunction, which was associated with an increase in GCN5L1 expression and an increase cardiac fatty acid oxidation rates. Weight loss due to dietary switch to LF diet was associated with a decrease in acetylation of LCAD, a decrease in GCN5L1 expression and a decrease in fatty acid oxidation rates. This suggests that weight loss favourably decreased LCAD acetylation in hearts from obese heart failure mice. It should be recognized, however, that there is a controversy in the literature whether acetylation of LCAD or other fatty acid oxidation enzymes is activating or inhibitory. Hirschey \textit{et al} demonstrated that in hepatocytes, deacetylation of the fatty acid oxidative enzyme LCAD increases fatty acid oxidation rates (36). In contrast, Zhao \textit{et al} showed that acetylation of β-hydroxyacyl CoA dehydrogenase, another enzyme involved in fatty acid oxidation, results in its activation (38). Nasrin \textit{et al} observed increased fatty acid oxidation in culture hepatocytes that had increased acetylation due to a decrease in SIRT4 expression (57). Moreover, fetal offspring of Japanese
macaques fed a HF diet during pregnancy had increased H3 acetylation in the liver compared to that of macaques fed a low fat diet (58). A recent study reported increased fatty acid oxidation in skeletal muscle of SIRT3KO mice (37). We also recently observed a positive correlation between acetylation status of LCAD and fatty acid oxidation rates in the obese mice and in SIRT3 KO mice (39).

An interesting finding from this study is that weight loss in obese heart failure mice was associated with a decrease in cardiac hypertrophy. We therefore examined what effect weight loss had on the hypertrophic signalling pathway in the hearts of obese heart failure mice. Inhibition of FOXO and the decrease in downstream atrogin-1 expression have been implicated in HF diet induced cardiac hypertrophy (59). On the other hand, activation of FOXO has been shown to promote cardiac (60) and skeletal muscle atrophy (61). In several tissues, FOXO1 and 3 can be regulated by SIRT1 deacetylation (30-34), with deacetylation of FOXO repressing its activity (32,33). In obese mice with heart failure we observed a significant increase in SIRT1 expression that was associated with decreased acetylation and inhibition of FOXO1 and a decreased atrogin-1 expression. Atrogin-1 is associated with skeletal muscle atrophy and therefore decreases in atrogin-1 also contribute to cardiac hypertrophy. We also observed that switching to LF diet in obese heart failure mice resulted in weight loss that was associated with a decrease in SIRT1, an increase in acetylation and activation of FOXO1 and a subsequent increase in atrogin-1 expression. While a HF diet could decrease SIRT1 expression, how SIRT1 expression is reduced by LF diet in HF AAC LF mice is unclear. We speculate that either a switch to a LF diet (with subsequent body weight changes) or the presence of pressure overload due to abdominal aortic could have altered SIRT1 expression in the HF AAC LF mice.
Another pathway involved in cardiac hypertrophy is protein kinase C (PKC). PKC activity is increased during the development of heart failure (62). In a pressure overload induced model of heart failure in guinea pigs, constriction of the descending thoracic aorta was accompanied by increase in PKC α expression during decompensated cardiac hypertrophy (63). Moreover, PKC is also involved in cardiac hypertrophy. PKC is hypothesized to modulate cardiac hypertrophy by phosphorylation of transcription factors controlling expression of hypertrophic genes. Among these transcription factors found to be modulated by PKC in agonist stimulated cardiomyocytes are c-jun and fos (64). PKC also promotes phosphorylation of mTOR and P70S6K resulting in activation of the cardiac hypertrophic pathways (65). We found that cardiac hypertrophy was associated with activation of mTOR and P70S6K in the obese heart failure mice. Weight loss in these mice resulted in activation of AMPK, resulting in inhibition of the mTOR and P70S6K pathway that led to a decrease in cardiac hypertrophy. These results are consist with a previous study showing that AMPK activation, with AICAR could reduce cardiac hypertrophy induced by a transverse aortic constriction in rats (66). In addition, resveratrol, an AMPK activator also reduced cardiac hypertrophy in the spontaneously hypertensive rats (67). In addition to its effect on mTOR, activation of AMPK can also activate P38MAPK (35) that can increase atrogin-1 independent of FOXO (68,69). Thus, it is possible that increased SIRT1 mediated the increased hypertrophy in obese heart failure mice, while activation of AMPK and FOXO1 blunted the hypertrophic response following LF diet induced weight loss.

It was interesting to note that the results of our study are in contrast to that of the clinical studies suggesting obesity paradox. Purposeful (intentional) weight loss resulted in improvement in cardiac function (15,16). We can therefore speculate that our mice were morbidly obese to the
point that obesity was a risk rather than being protective. Moreover, one study has shown a higher mortality in obese patients with systolic heart failure in comparison to obese patients with diastolic heart failure (12). Since, the mice in our study had systolic heart failure in addition to diastolic dysfunction, it is possible that weight loss may have improved cardiac function.

It should also be pointed out that clinical studies examining obesity paradox have not excluded patients with unintentional weight loss due to cachexia of heart failure. In such scenarios, being obese may offer the metabolic reserve improving survival (70). Therefore, carefully controlled studies excluding patients with cachexia (or unintentional weight loss) upon entry into clinical trials may help resolve the obesity paradox. Furthermore, identifying the best index of obesity that could predict survival in obese patients with heart failure would add value.

In summary, we show that weight loss in obese heart failure mice can improve diastolic function, which is associated with a decrease in cardiac hypertrophy possibly due to inhibition of SIRT1 and activation of AMPK. Furthermore, improved insulin sensitivity due to suppression of SOCS3 could also have contributed to the observed increase in insulin-stimulated glucose oxidation. While alterations in cardiac hypertrophy, metabolism and insulin sensitivity could have improved cardiac function in obese heart failure mice, the primary pathway remains to be determined and is a limitation of our study. Therefore, lowering body weight in obese mice with heart failure has a number of beneficial effects, including improving cardiac hypertrophy, cardiac function and restoring cardiac insulin sensitivity. Overall, our findings demonstrate that increased weight was not associated with improved outcomes in mice with heart failure. These
findings, based upon surrogate but clinically important outcomes, do not support the existence of an ‘obesity paradox’.
Acknowledgements:

Dr. Gary D. Lopaschuk is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

S.S. was involved in study design, conduct of experiments, analysis of results, writing of the manuscript for submission. O.A. was involved in the conduct of experiments, and analysis of results. L.Z. was involved in performing additional experiments during the revision of this manuscript. J.J. was involved in the study design and critical revisions to the draft of the manuscript. C.S.W. was involved in the conduct of experiments. A.F. was involved in the conduct of experiments. R.P. was involved in study design, provided critical revisions to the manuscript and funding for this study. D.J. was involved in study design, provided critical revisions to the manuscript and funding for the study. A.S. was involved in study design, provided critical revisions to the manuscript and funding for this study. G.L. was involved in study design, provided critical revisions to the manuscript and funding for this study.

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Conflicts of Interest: None
References:


34. Sundaresan NR, Pillai VB, Wolfgeher D, et al. The deacetylase SIRT1 promotes membrane localization and activation of Akt and PDK1 during tumorigenesis and cardiac hypertrophy. Sci Signal 2011.


Figure legends:

**Figure 1. Effect of a low fat diet on weight gain and glucose tolerance in obese mice with heart failure**

A: Body weight (g) over the 18 wk study protocol in LF sham, HF sham, HF AAC HF, and HF AAC LF groups.  B: Fat mass (g) and C: Lean mass (g) at the end of the 18 wk study protocol in LF sham, HF sham, HF AAC HF, and HF AAC LF groups.  D: Glucose tolerance at 18 wk in LF sham, HF sham, HF AAC HF, and HF AAC LF groups.

** P<0.01, *** P<0.001 vs. LF Sham; † P<0.05, †† P<0.01 vs. HF AAC HF. Values represent mean ± SEM (n=5-6).

**Figure 2: Effects of weight loss on in vivo cardiac function in obese mice with heart failure**

Indices of diastolic function assessed by A: E/A ratio, B: E'/A' ratio, C: E/E' ratio. D: Systolic function as assessed by echocardiographic %EF.  E: SERCA2a/α-tubulin expression as a factor regulating cardiac contractile function. * P<0.05, ** P<0.01 vs. baseline or LF Sham as appropriate; † P<0.05 vs. HF AAC HF at the same time point; ‡ P<0.05 vs. HF AAC HF. Values represent mean ± SEM (n=5-6).

**Figure 3: Effects of weight loss and mechanisms contributing to cardiac hypertrophy in obese mice with heart failure**

Indices of cardiac hypertrophy at the end of the 18 wk protocol assessed by A: in vivo echocardiography, Corrected LV mass (mg), and B: in vitro method, Heart weight / tibia length (mg/mm). Molecular markers of cardiac hypertrophy measured in heart tissue after 18 weeks in...
LF sham, HF sham, HF AAC HF, and HF AAC LF groups. C: α-Skeletal actin/α-tubulin, D: atrial natriuretic peptide (ANP)/α-tubulin. Cellular signalling pathways contributing to cardiac hypertrophy assessed at the end of the 18 wk protocol in LF sham, HF sham, HF AAC HF, and HF AAC LF groups. E: Expression of SIRT 1/β-actin, F: content of acetylated FOXO1, G: expression of atrogin-1/β-actin, H: content of P-p38 MAPK/p38 MAPK. * P<0.05 vs. baseline or LF Sham; † P<0.05 vs. HF AAC HF; †† P<0.01 vs. HF Sham or HF AAC HF (as appropriate); ‡ P<0.05 vs. HF AAC HF. Values represent mean ± SEM (n=5-6).

**Figure 4: Mechanisms contributing to prevention of cardiac hypertrophy in obese mice with heart failure**

Cellular signalling pathways contributing to cardiac hypertrophy assessed at the end of the 18 wk protocol in LF sham, HF sham, HF AAC HF, and HF AAC LF groups. A: content of P-AMPK/AMPK, B: PKC-α expression/β-actin, C: content of P-mTOR/m-TOR, D: content of P-p70S6K/p70S6K. * P<0.05, *** P<0.001 vs. LF Sham; † P<0.05; †† P<0.01 vs. HF AAC HF; Values represent mean ± SEM (n=5-6).

**Figure 5: Effects of weight loss on cardiac energy metabolism in obese mice with heart failure**

Cardiac energy metabolism assessed at the end of the 18 wk study protocol in isolated hearts from LF sham, HF sham, HF AAC HF, and HF AAC LF groups A: glucose oxidation (µmol g dry wt⁻¹ min⁻¹), B: palmitate oxidation (µmol g dry wt⁻¹ min⁻¹), C: ATP production (µmol g dry wt⁻¹ min⁻¹), and D: TCA cycle activity (µmol g dry wt⁻¹ min⁻¹). * P<0.05, ** P<0.01 vs. respective group W/O insulin; †† P<0.01 vs. HF AAC HF W/ Insulin; ‡‡ P<0.01 vs. glucose
oxidation or palmitate oxidation HF AAC HF W/ Insulin respectively. Values represent mean ± SEM (n=5-6).

**Figure 6: Effects of weight loss on the cardiac insulin signaling in obese mice with heart failure**

Insulin signaling assessed at the end of the 18 wk study protocol in isolated hearts from LF sham, HF sham, HF AAC HF, and HF AAC LF groups A: P-Akt/Akt, B: P-GSK3β/GSK3β, C: m-GLUT4/Caveolin-3, D: m-GLUT1/Caveolin-3, E: SOCS3/β-actin, and F: P-STAT3/STAT3. *P<0.05, **P<0.01 vs. LF Sham; † P<0.05 vs. HF Sham; ‡ P<0.05 vs. HF AAC HF. Values represent mean ± SEM (n=5-6).

**Figure 7: Effects of weight loss on acetylation control of fatty acid oxidation in obese mice with heart failure**

Acetylation control of fatty acid oxidation assessed at the end of the 18 wk study protocol in isolated hearts from LF sham, HF sham, HF AAC HF, and HF AAC LF groups. A: GCN5L1 expression/β-actin, B: SIRT3/β-actin, C: LCAD expression/β-actin, D: content of acetylated LCAD/LCAD, E: LCAD activity (nmol mg protein⁻¹ min⁻¹), and F: correlation between palmitate oxidation and the content of acetylated LCAD/LCAD. *P<0.05, **P<0.01 vs. LF Sham; † P<0.05 vs. HF AAC HF; ‡ P<0.05 vs. HF Sham; Values represent mean ± SEM (n=5-6).

**Figure 8. Schematic diagram showing alterations in various pathways in obese mice with heart failure**
Schematic diagram summarizing the various pathways that regulate cardiac metabolism, insulin sensitivity and cardiac hypertrophy in obese mice with heart failure and the effect of weight loss due to diet change.
Figure 1.

A

```
--- LF Sham  --- HF Sham  --- HF AAC HF  --- HF AAC LF
```

```
AAC / Sham Surgery  Switch Diet
```

```
0  1  2  3  4  5  6  7  8  9  10 11 12 13 14 15 16 17 18
```

```
Body Weight (g)
```

```
Time (Weeks)
```

B

```
0  5  10  15  20
```

```
Fat Mass (g)
```

```
LF Sham  HF Sham  HF AAC HF  HF AAC LF
```

```
***  ***
```

```
† †
```

C

```
0  10  20  30  40
```

```
Lean Mass (g)
```

```
LF Sham  HF Sham  HF AAC HF  HF AAC LF
```

D

```
--- LF Sham  --- HF Sham  --- HF AAC HF  --- HF AAC LF
```

```
Blood Glucose (mmol/L)
```

```
0  5  10  15  20  25
```

```
Time (min)
```

```
0  15  30  60  90  120
```

```
***  **  *  +
```

```
† ††
```

38
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.
Figure 7.
Figure 8.

Obesity and Heart Failure

- **SIRT 1**
- **Acetylation FOXO1**
- **PKC-α**
- **FOXO1**
- **P-mTOR**
- **P-P70S6K**

- **Insulin Resistance**
  - **P-STAT3**
  - **P-Akt**
  - **SOCS-3**
  - **P-GSK3β**

- **Palmitate Oxidation**

Effect of Low Fat Diet and Weight Loss

- **SIRT 1**
- **Acetylation FOXO1**
- **AMPK**
- **PKC-α**
- **FOXO1**
- **P-PSIMAPK**
- **P-mTOR**
- **P-P70S6K**

- **Insulin Sensitivity**
  - **P-STAT3**
  - **P-Akt**
  - **SOCS-3**
  - **P-GSK3β**

- **Palmitate Oxidation**

- **LCAD Activity**

- **Ac-LCAD**

- **GCNSL1**
Online Appendix

Lowering Body Weight in Obese Mice with Diastolic Heart Failure Improves Cardiac Insulin Sensitivity and Function: Implications for the Obesity Paradox

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Running title: Cardiac insulin resistance in obesity

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Supplemental Figure 1: Effects of weight loss on the cardiac fatty acid and glucose oxidation pathways in obese mice with heart failure

A: Malonyl-CoA content at the end of the 18 wk study protocol in isolated hearts from LF sham, HF sham, HF AAC HF, and HF AAC LF groups. B: content of P-ACC/ACC, C: expression of MCD/β-actin, D: expression of P-PDH/PDH and E: expression of PDK4/β-Actin at the end of the 18 wk study protocol in isolated hearts from LF sham, HF sham, HF AAC HF, and HF AAC LF groups. *P<0.05 vs. HF AAC HF. Values represent mean ± SEM (n=5-6).
Supplemental Figure 2

**Supplemental Fig 2: Effects of weight loss on the cardiac insulin signaling in obese mice with heart failure**

Insulin signalling assessed at the end of the 18 wk study protocol in isolated hearts from LF sham, HF sham, HF AAC HF, and HF AAC LF groups A: P-Akt/β-Actin, B: GLUT4/β-Actin, C: GLUT1/α-Tubulin. *P<0.05 vs. LF Sham; ‡P<0.05 vs. HF AAC HF. Values represent mean ± SEM (n=4-5).
Supplemental Table 1. Echocardiographic assessment of cardiac function in mice fed a low or high fat diet for 18 weeks.

<table>
<thead>
<tr>
<th></th>
<th>LF Sham</th>
<th>HF Sham</th>
<th>HF AAC HF</th>
<th>HF AAC LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>490 ± 19</td>
<td>496 ± 24</td>
<td>501 ± 21</td>
<td>481 ± 17</td>
</tr>
<tr>
<td>E-wave (mm/s)</td>
<td>750 ± 17</td>
<td>720 ± 14</td>
<td>710 ± 18</td>
<td>725 ± 16</td>
</tr>
<tr>
<td>A-wave (mm/s)</td>
<td>517 ± 15</td>
<td>572 ± 18**</td>
<td>348 ± 19**</td>
<td>443 ± 10††</td>
</tr>
<tr>
<td>E/A Ratio</td>
<td>1.5 ± 0.1</td>
<td>1.9 ± 0.1*</td>
<td>2.0 ± 0.1**</td>
<td>1.6 ± 0.05†</td>
</tr>
<tr>
<td>E' (mm/s)</td>
<td>29.9 ± 2.6</td>
<td>24.9 ± 2.2</td>
<td>18.3 ± 2.1</td>
<td>26.7 ± 2.8</td>
</tr>
<tr>
<td>A' (mm/s)</td>
<td>22.2 ± 1.9</td>
<td>22.6 ± 2.0</td>
<td>19.9 ± 2.3</td>
<td>23.2 ± 2.1</td>
</tr>
<tr>
<td>E'/A' Ratio</td>
<td>1.35 ± 0.1</td>
<td>1.09 ± 0.05</td>
<td>0.91 ± 0.05**</td>
<td>1.15 ± 0.1†</td>
</tr>
<tr>
<td>E/E' Ratio</td>
<td>25.1 ± 2.6</td>
<td>28.9 ± 3.2</td>
<td>38.8 ± 2.5**</td>
<td>27.2 ± 1.7†</td>
</tr>
<tr>
<td>LV Mass (mg)</td>
<td>95.5 ± 4.1</td>
<td>117.4 ± 4.4*</td>
<td>149.2 ± 5.7***</td>
<td>122.0 ± 3.0**</td>
</tr>
<tr>
<td>LVPWD (mm)</td>
<td>0.77 ± 0.02</td>
<td>0.87 ± 0.05</td>
<td>0.97 ± 0.04**</td>
<td>0.83 ± 0.02</td>
</tr>
<tr>
<td>LV EF (%)</td>
<td>54.8 ± 4.4</td>
<td>57.7 ± 3.1</td>
<td>45.3 ± 2.3*</td>
<td>51.2 ± 1.6</td>
</tr>
<tr>
<td>LV FS (%)</td>
<td>28.5 ± 2.8</td>
<td>30.3 ± 2.1</td>
<td>22.6 ± 2.0†</td>
<td>24.8 ± 1.2</td>
</tr>
</tbody>
</table>

LF, low fat; HF, high fat; AAC, abdominal aortic constriction.
E-wave, peak early transmitral inflow mitral E velocity; A-wave, transmitral inflow velocity due to atrial contraction; E', early diastolic mitral inflow tissue doppler velocity; A', transmitral inflow tissue doppler velocity due to atrial contraction; LV Mass, left ventricular mass; LVPWD, LV posterior wall diameter; LV EF, LV ejection fraction; LVFS, Left ventricular fractional shortening. Results are presented as mean±S.E.M.
* p<0.05; **p<0.01, ***p<0.001 compared to LF Sham; †p<0.05; ††p<0.01 compared to HF AAC LF.
Supplemental Table 2. In vitro cardiac function of hearts in working heart mode from mice fed a low or high fat diet for 18 weeks.

<table>
<thead>
<tr>
<th></th>
<th>LF Sham</th>
<th>HF Sham</th>
<th>HF AAC HF</th>
<th>HF AAC LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (bpm)</td>
<td>274.4 ± 4.7</td>
<td>287.3 ± 2.2</td>
<td>282.7 ± 6.0</td>
<td>287.1 ± 6.7</td>
</tr>
<tr>
<td>Rate Pressure Product (bpm·mmHg)</td>
<td>20.4 ± 0.3</td>
<td>19.4 ± 0.4</td>
<td>18.4 ± 0.3*</td>
<td>19.9 ± 0.4†</td>
</tr>
<tr>
<td>Cardiac Output (ml/min)</td>
<td>11.7 ± 0.6</td>
<td>11.4 ± 0.8</td>
<td>10.9 ± 0.2</td>
<td>12.5 ± 0.9</td>
</tr>
<tr>
<td>Cardiac Work (joules·g⁻¹·min⁻¹)</td>
<td>2.6 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>3.1 ± 0.3†</td>
</tr>
</tbody>
</table>

LF, low fat; HF, high fat; AAC, abdominal aortic constriction. Values represent mean ± SEM. *p<0.05 compared to LF Sham; †p<0.05 compared to HF AAC HF.