Recent studies suggest improved outcomes and survival in obese heart failure patients (i.e., the obesity paradox), although obesity and heart failure unfavorably alter 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 (HF) diet (60% kcal from fat) for 4 weeks, following which an abdominal aortic constriction (AAC) was produced. Four weeks post-AAC, mice were switched to a low-fat (LF) diet (12% kcal from fat; HF AAC LF) or maintained on an HF (HF AAC HF) for a further 10 weeks. After 18 weeks, HF AAC LF mice weighed less than HF AAC HF mice. 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 with HF AAC HF mice. Insulin-stimulated glucose oxidation was increased in hearts from HF AAC LF mice, compared with HF AAC HF mice. 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.

Obesity is a recognized risk factor for heart failure (1,2). Obesity is associated with left ventricular (LV) hypertrophy and dilatation, features that are known to precede the development of overt heart failure (3,4). For every increase in 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 favorable 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 HF to low-fat (LF) 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 HF to LF diet in the obese mice with heart failure had on diastolic function, cardiac hypertrophy, and cardiac energy metabolism.

RESEARCH DESIGN AND 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 weeks of age) were randomly assigned to be fed either a standard chow/LF diet (12% kcal from fat) or a HF diet (60% kcal from fat). Four weeks later, mice in both groups were anesthetized with 0.75% isoflurane and underwent sham or AAC surgical procedure (24). Briefly, a 2-cm mediolateral 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 AAC surgery were continued on their respective diet for a further 4-week period. At 4 weeks postsurgery (8 weeks 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 subgroup of mice that was subsequently randomized to be fed the LF diet (HF AAC LF; 36.5 ± 3.7 vs. 38.1 ± 4.6 mmHg). Mice in the HF AAC group were then randomly divided to either continuing on an HF diet or switching to an LF diet for a further 10-week period. The HF sham and LF sham groups were also continued on their respective diets for a further 10-week 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 weeks of the protocol. After an overnight fast for 16 h, a fasting blood glucose sample was obtained. Subsequently, mice were challenged with glucose (2 g/kg body weight) orally. Blood glucose was measured at 15, 30, 60, 90, and 120 min after glucose administration using an ACCU-CHEK Aviva (Roche Diagnostics) glucometer.

MRI

Fat and lean mass composition was analyzed after 18 weeks of the feeding protocol using EchoMRI QMNR 4-in-1 Whole Body Composition Analyzer (Echo Medical Systems, Houston, TX).

Echocardiography and Tissue Doppler Imaging

Echocardiography was performed using a VisualSonics Vevo 770 high-resolution echocardiography imaging system equipped with a 30-MHz transducer (RMV-707B; VisualSonics, Toronto, Ontario, Canada) (26). Echocardiographic analyses of in vivo cardiac function were carried out at baseline (4 weeks) and 6, 8, 14, and 18 weeks 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 LV wall thickness, LV end-diastolic diameter, and LV end-systolic diameter. LV ejection fraction (%EF) and fractional shortening were calculated to assess systolic function. Tissue Doppler imaging 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. Tissue Doppler imaging was used 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 weeks of the treatment protocol, mice were killed with sodium pentobarbital. Hearts were quickly excised after a thoracic incision, cannulated, and perfused as isolated working preparations as described previously (24). Hearts were perfused with Krebs-Henseleit solution containing 2.5 mmol/L CaCl2, 5 mmol/L [U-14C]glucose, and 0.8 mmol/L [9,10-3H]palmitate prebound 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 14CO2 and 3H2O produced from the oxidation of [U-14C]glucose and [9,10-3H] palmitate, respectively, as described previously (24). At the end of the perfusion, hearts were quickly frozen with tongs cooled to the temperature of liquid N2.

**Determination of Cardiac Triacylglycerol**
Triacylglycerol (TG) 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 mmol/L 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 analysis, as described previously (28). For short-chain CoA ester analysis, 10 μL of the sample was run through an Ascentis Express C18 column (10 cm × 2.1 mm and 2.7 μm particle size; Supelco Oakville, Ontario, 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 mmol/L NaH2PO4 and buffer B (250 mmol/L NaH2PO4 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; and 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 0.05 mol/L Tris-HCl, 10% glycerol, 1 mmol/L EDTA, 0.02% Brij-35, and 1 mmol/L dithiothreitol in the presence of protease and phosphatase inhibitors (Sigma-Aldrich). 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 in a sample preparation buffer containing 0.062 mol/L Tris-HCl, 10% glycerol, 0.003% bromphenol blue, 5% 2-β-mercapto-ethanol, 2% SDS, and 6 mol/L 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, pyruvate dehydrogenase (PDH), P-PDH, PDH kinase 4 (PDK4), GSK3β, P-GSK3β, SIRT1, FOXO1, Atroglin-1, P-38 MAPK, P-P38 MAPK (Cell Signaling Technology Inc., Danvers, MA), acetyl-lysine (Millipore Inc., Billerica, MA), SIRT3, and Glut4, alpha skeletal actin, ANP, and long chain acyl Coa dehydrogenase (LCAD; Abcam Inc., Toronto, Ontario, Canada). GCN5L1 was generously provided by Dr. M.N. Sack (National Institutes of Health, Bethesda, MD). Membranes were incubated with the appropriate secondary antibodies (Santa Cruz Biotechnologies, Santa Cruz, CA) for 1 h. Enhanced chemiluminescence (Bio-Rad Inc., Hercules, CA) system was used for band detection. The intensity of band signals was analyzed by Quantity One software (4.4.0).

**Immunoprecipitation**
For immunoprecipitation, 100 μg of the total heart lysate was used. Lysates were incubated with acetyl-lysine antibodies (3 μg/100 μg lysate) overnight at 4°C, and 50 μL of protein A-agarose beads were added to each sample and incubated on a rotator for 4 h at 4°C. After 4 h, samples were washed 3× with 100 μL of homogenization buffer containing 3 mol/L NaCl and centrifuged at 16,000 g 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 mean ± SEM. Data were analyzed by one- or two-way ANOVA. When ANOVA revealed differences, data sets were compared by Bonferroni multiple-comparisons posttest. P < 0.05 was deemed significant.

**RESULTS**

**Obesity Heart Failure Model**
Following 4 weeks 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 diet. At the 8-week time point, mice fed HF weighed significantly more than mice fed LF that were subjected to the sham surgical procedure (34.8 ± 2.0 [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 weeks. After 18 weeks,
the HF sham and HF AAC HF groups had significantly higher body weight compared with the LF sham group, while the HF AAC LF group did not gain weight, and ultimately had 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 with LF sham, while the HF AAC LF group had decreased fat mass compared with HF sham and HF AAC 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 differences in fat mass. Impaired glucose tolerance had developed in HF mice at 8 weeks as area under the curve (mmol/L · min) was significantly increased in both HF sham and HF AAC HF compared with 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 weeks, glucose tolerance (mmol/L · min) was improved in HF AAC LF mice compared with 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 HF to LF 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-week study protocol (Supplementary Table 1).
HF mice subjected to an AAC, diastolic dysfunction occurred as early as 2 weeks after the procedure (i.e., 6 weeks 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 an LF diet at 8 weeks (HF AAC LF), a significant improvement in diastolic function was seen (Fig. 2A and Supplementary 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 weeks post-AAC in the HF AAC HF mice, demonstrating diastolic dysfunction. Diastolic dysfunction was attenuated in HF AAC LF mice (Fig. 2B and C). Systolic function (%EF) was normal in LF sham and HF sham mice (Supplementary Table 1). AAC induced a significant reduction in %EF compared with baseline in HF mice (Fig. 2D). In the HF AAC LF mice, a significant improvement in %EF was observed at the end of the 18-week study period (Fig. 2D and Supplementary Table 1). Furthermore, as alterations in calcium handling can affect cardiac contractile function, we measured SERCA2a expression in these

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**Figure 2**—Effects of weight loss on in vivo cardiac function in obese mice with heart failure. Indices of diastolic function assessed by E/A ratio (A), E'/A' ratio (B), and E/E' ratio (C). 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).
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 B) in the HF AAC 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 with 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 HF to LF 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 expression in these hearts. Both markers were significantly increased in the HF AAC HF hearts compared with LF sham and reduced significantly in the HF AAC LF hearts (Fig. 3C and D).

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 protein kinase C (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 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 with 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 with 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-week study. In hearts from the LF sham group, insulin increased glucose oxidation rates (Fig. 5A) while inhibiting 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 inhibition of fatty acid oxidation (Fig. 5B). In hearts from the HF AAC HF group, fatty acid oxidation dominated as a source of ATP production (Fig. 5C) and tricarboxylic acid 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. 5C and D). While cardiac fatty acid oxidation rates can be regulated by malonyl CoA, glucose oxidation rates can be regulated by PDH, 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-week study protocol. Malonyl CoA levels were similar in LF sham, HF sham, and HF AAC HF mouse hearts (Supplementary Fig. 1A). HF AAC LF mouse hearts had significantly increased cardiac malonyl CoA content (Supplementary Fig. 1A). This was associated with a significant decrease in phosphorylation (inhibition) of acetyl CoA carboxylase, the enzyme responsible for malonyl CoA synthesis (Supplementary Fig. 1B). In contrast, the expression of malonyl CoA decarboxylase (which is responsible for malonyl CoA degradation) remained unchanged (Supplementary 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 PDK4 expression (Supplementary Fig. 1D and E). 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 with 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 HF to LF 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
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-week protocol assessed by in vivo echocardiography (corrected LV mass [mg]) (A) and in vitro method (HW/TL [mg/mm]) (B). 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/α-tubulin. Cellular signaling pathways contributing to cardiac hypertrophy assessed at the end of the 18-week protocol in LF sham, HF sham, HF AAC HF, and HF AAC LF groups. E: Expression of SIRT1/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). Ac, acetylated; ANP, atrial natriuretic peptide.
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 normalized 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 AAC LF hearts compared with HF AAC HF mouse 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 HF to LF on Acetylation Control of Fatty Acid Oxidation in Obese Mice With Heart Failure

Recent studies have implicated posttranslational 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, 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 with LF sham hearts (Fig. 7A). The HF AAC LF hearts had significantly lower GCN5L1 expression compared with 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 with HF AAC HF mouse hearts (Fig. 7C). In parallel with the increase in GCN5L1 expression in the HF AAC HF mouse, 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 (Fig. 8). 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 HF to LF 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 HF to LF restored cardiac insulin sensitivity and switched energy substrate metabolism back toward 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 antihypertrophic signaling pathways. Combined, our data clearly demonstrate 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 an 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 signaling. 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 with LF sham hearts. GSK3β can be phosphorylated by PKC, PKA, as well as by 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.

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-week study protocol in isolated hearts from LF sham, HF sham, HF AAC HF, and HF AAC LF groups. A: Glucose oxidation (nmol·g dry wt⁻¹·min⁻¹). B: Palmitate oxidation (nmol·g dry wt⁻¹·min⁻¹). C: ATP production (μmol·g dry wt⁻¹·min⁻¹). D: Tricarboxylic acid cycle activity (μmol·g dry wt⁻¹·min⁻¹). *P < 0.05, **P < 0.01 vs. respective group without insulin; ††P < 0.01 vs. HF AAC HF with insulin; ‡‡P < 0.01 vs. glucose oxidation or palmitate oxidation HF AAC HF with insulin, respectively. Values represent mean ± SEM (n = 5–6). TCA, tricarboxylic acid; W/, with; W/O, without.
While leptin is known to be increased in mice fed an HF diet in comparison with 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 signaling 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.

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-week 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. 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).
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, carnitine palmitoyltransferase (CPT)-1b−/− mice that partially lack 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).

![Figure 7](image-url)
While maintaining adequate fatty acid oxidation may be important in heart failure, our data suggest 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 favorably decreased LCAD acetylation in hearts from obese heart failure mice. It should be recognized, however, that there is a controversy

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**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. Ac, acetylated.
increases fatty acid oxidation rates. In contrast, Zhao et al. (36) demonstrated that in hepatocytes, deacetylation of the fatty acid oxidative enzyme LCAD increases fatty acid oxidation rates. In contrast, Zhao et al. (36) showed that acetylation of β-hydroxyacyl CoA dehydrogenase, another enzyme involved in fatty acid oxidation, results in its activation. Nasrin et al. (57) observed increased fatty acid oxidation in culture hepatocytes that had increased acetylation due to a decrease in SIRT4 expression. Moreover, fetal offspring of Japanese macaques fed an HF diet during pregnancy had increased H3 acetylation in the liver compared with that of macaques fed an LF 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 signaling 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 an 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 an LF diet (with subsequent body weight changes) or the presence of pressure overload due to abdominal aortic constriction could have altered SIRT1 expression in the HF AAC LF mice.

Another pathway involved in cardiac hypertrophy is 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, c-jun and fos are associated with increased hypertrophy. 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 an 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 an LF diet (with subsequent body weight changes) or the presence of pressure overload due to abdominal aortic constriction could have altered SIRT1 expression in the HF AAC LF mice.
Therefore, lowering body weight in obese mice with heart failure has a number of beneficial effects, including improving cardiac hypertrophy and 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.

Funding. This study was funded by a grant to G.D.L. from the Heart and Stroke Foundation of Canada and by a grant from the University Hospital Foundation. G.D.L. is supported by an Alberta Heritage Foundation for Medical Research Scientist Award. S.S. received a fellowship from Alberta Innovates Health Solutions.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. S.S. was involved in study design, conduct of experiments, analysis of results, and writing of the manuscript for submission. O.A.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.S.J. was involved in the study design and critical revisions to the draft of the manuscript. C.S.W. and A.F. were involved in the conduct of experiments and provided critical revisions to the manuscript and funding for this study. G.D.L. is the guarantor of this work and, as such, had full access to all the data in the data analysis and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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