Mechanical Adaptations of Skinned Cardiac Muscle in Response to Dietary-Induced Obesity During Adolescence in Rats

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Abstract

Childhood obesity is a major risk factor for heart disease during adulthood, independent of adulthood behaviours. Therefore, it seems that childhood obesity leads to partly irreversible decrements in cardiac function. Little is known about how obesity during maturation affects the mechanical properties of the heart. The purpose of this study was to evaluate contractile properties in developing hearts from animals with dietary-induced obesity (high-fat high-sucrose diet (HFHS)). We hypothesized that obesity induced during adolescence results in decrements in cardiac contractile function.

Three-week old rats (n=16) were randomized into control (chow) or dietary-induced obesity (HFHS) groups. Following 14 weeks on the diet, skinned cardiac trabeculae fibre bundle testing was performed to evaluate active and passive force, maximum shortening velocity, and calcium sensitivity.

Rats in the HFHS group had significantly larger body mass and total body fat percentage. There were no differences in maximal active or passive properties of hearts between groups. Hearts from HFHS group rats had significantly slower maximum shortening and lower calcium sensitivity than controls.

Decreased shortening velocity and calcium sensitivity in hearts of obese animals may constitute increased risk of cardiac disease in adulthood.

Novelty Bullets

- Cardiac muscle from animals exposed to an obesogenic diet during development had lower shortening velocity and calcium sensitivity than those from animals fed a chow diet.
- These alterations in mechanical function may be a mechanism for the increased risk of cardiac disease observed in adulthood.

Keywords

- Adolescent Obesity
- Dietary-induced Obesity
- High-fat High-sucrose Diet
- Cardiac Adaptations
- Cardiac Health
- Skinned Fibres

Introduction

Cardiovascular disease (CVD) is a leading cause of death and disability, and accounts for approximately one third of all deaths in Canada (Smith 2009). One of the major risk factors for developing CVD is obesity. Death from CVD is 2-3 times more likely in individuals with a body mass index (BMI) ≥ 35 kg/m² than individuals with a normal (18.5-24.9 kg/m²) BMI (Calle et al. 1999). Large-scale epidemiological data suggest a 30% increase in risk of CVD associated mortality with each 5 kg/m² increase in BMI (Selmer and Tverdal 1995). Even when considered independently of other common co-morbidities (dyslipidemia, hypertension), obesity doubles the risk for heart failure (Kenchiah et al. 2002; Wong and Marwick 2007).

With the prevalence of obesity increasing exponentially worldwide, particular concern has been placed on the rise in obesity rates in children and adolescents, and the unique challenges obesity poses to normal development. It is estimated that 35% and 26% of youth in North America are overweight and obese, respectively (Skinner et al. 2018). This prevalence is expected to further increase and has been identified as a major health concern. Further, higher levels of body fat in childhood strongly predict body fat in adulthood, further exacerbating obesity-related effects over the lifespan (Goran 2001).

Childhood obesity has been shown to negatively affect health during childhood and adolescence, as well as later in life (Franks et al. 2010). These effects include a greater risk for cardiovascular events compared to non-obese youth, as well increased morbidity over the lifespan (Goran 2001; Must et al. 1992). In a longitudinal study of children, starting at a mean age of 11.3 $\pm$ 3.7 years, obesity was associated with a significantly greater rate of death during adulthood (before age 55) (Franks et al. 2010). In another study, 3000 school-aged children were assessed at baseline and followed longitudinally (Dearborn et al. 1938). Forty and sixty years later, a follow-

up study considered 500 of the participants (both alive and deceased) and identified a 2-fold greater risk of mortality from cardiovascular disease in those overweight as children (Must et al. 1992). These effects were independent of body composition during adulthood, indicating that childhood obesity is an independent risk factor, regardless of adult behaviour. These results underscore the importance of maintaining a healthy body composition during childhood and adolescence, and they suggest that there may be persistent maladaptations in children with obesity.

Evidence of the effects of obesity on cardiac muscle mechanics is limited and is based primarily from studies on leptin-deficient obese animal models. Leptin is a hormone secreted by adipocytes that acts to regulate adipose tissue mass and body weight (Friedman and Halaas 1998), and its deficiency in rodents has been shown to result in severe obesity (Ross et al. 2004). Previous studies compared intact cardiac fibres from 12-week old (Dong et al. 2006) and 9-week old (Li et al. 2006) leptin deficient obese mice and found decreased release and slower reuptake of intracellular calcium, and slower rates of shortening and relaxation in obese mice compared to control animals. Similarly, Relling et al. (2006) compared ventricular intact myocytes from 13-week old leptin deficient obese rats to control animals, and found depressed unloaded shortening velocities, and reduced release and slower reuptake of intracellular calcium. These three studies provide evidence that there are differences in the physiology of the cardiac muscle following leptin deficiency-induced obesity. However, since leptin plays an important role in regulating the immune system as well as facilitating satiety (Paz-Filho et al. 2012), it is unknown if the adaptations observed in these studies are in response to obesity, or in part due to systemic effects associated with the leptin deficiency. It is also not clear from these studies if there are mechanical adaptations of the heart muscle in addition to the physiological differences.

The mechanical consequences of obesity during childhood on the heart muscle are not well known. Additionally, it is not known why obesity during childhood leads to sustained maladaptation later in life. It is conceivable that mechanical maladaptation during development may be a possible mechanism for the greater risk of cardiac events later in life. Therefore, the purpose of this study was to evaluate the differences between contractile properties in normally developing hearts (animals fed a chow diet), and those from animals with dietary-induced obesity (animals fed a high-fat high-sucrose diet) from post-weanling age through to adolescence. We hypothesized that obesity induced during adolescence and maturation would result in decrements in cardiac contractile function.

Method

Obesity Induction

Three-week old male Sprague-Dawley rats were randomized into two groups. The control group (n=8) was fed a standard chow diet (Lab Diets 5001) consisting of 30% protein, 13% fat, and 57% carbohydrates (3% of carbohydrates from sucrose). For the first 7 weeks, animals in the dietary-induced obesity group (n=8) were fed a high-fat high-sucrose diet (Dyets 103915) consisting of 24% protein, 20% fat, and 45% protein, and for the second half of the study weeks 8-14) the diet (Dyets 102412) was comprised of 15-20% protein, 40% fat, and 40-45% carbohydrates (>97% of carbohydrates from sucrose). Obesity was induced over a fourteen week period, a time frame that has been shown previously to result in obesity in adult rats (Collins et al. 2016, Collins et al. 2017; Rios et al. 2019). The diet began at weaning and was maintained through adolescence and into adulthood. Animals were housed individually at 21° C on a 12:12 light-dark cycle, and had access to water ad libitum. The study protocol was approved by The University of

Calgary Animal Care Committee and conformed to the Guide for the Care and Use of Laboratory Animals.

Body Composition

Rats were weighed weekly over the course of the intervention. Following the fourteen weeks of dietary obesity induction, animals were weighed to determine body mass, and scanned using Dual Energy X-ray Absorptiometry to determine body composition. The length of each animal's left tibia was measured via x-ray to account for differences in body size (Yin et al. 1982).

Tissue Isolation

While anesthetized, the rat's chest cavities were opened. Animals were euthanized by severing the aorta and vena cava and the heart was removed. Hearts were immediately flushed with relaxing solution and the aorta and vena cava were further dissected away. Hearts were weighed to determine heart mass, and were normalized to the left tibia length to control for body size, independent of body composition. The left ventricle was cut open and pinned, while 2-3 thin strips of trabeculae were sliced across the left ventricle wall and placed in relaxing solution on ice. After two hours in the relaxing solution, samples were transferred to a 50/50 (v/v) relaxing/glycerol solution and left overnight on ice in the fridge. The following morning, the muscle strips were transferred to a fresh 50/50 relaxing/glycerol solution and stored at -20 °C for three weeks before mechanical testing. All samples were tested within a week of being skinned. An additional strip of trabeculae was dissected and immediately flash frozen in liquid nitrogen and stored at -80 °C for biochemical analysis.

Mechanical Testing

On the day of mechanical testing, a strip of muscle was removed and placed in a relaxing solution where fibre bundles of approximately 100-300 μm in width and 1000-2000 μm in length were isolated manually under a Nikon SMZ1500 microscope. One end of the sample was pierced and glued with cellulose acetate to a hook connected to a length controller (Aurora Scientific Inc., Model 308, Ontario, Canada). The other end was similarly attached to the hook of a force transducer (Aurora Scientific Inc., Model 400A, Ontario, Canada), thereby allowing for simultaneous control of myocyte length and measurement of force. All experiments were performed at $\sim 15^\circ\text{C}$. The sample length was coarsely adjusted from slack until tension first developed. A He-Ne laser beam was then used to finely adjust average sarcomere length to a resting length of 2.2 μm . Following a three-minute rest period at 2.2 μm to allow for any stress-relaxation, the length and width of the sample, and the passive (resting) force were recorded. Cross-sectional area of the sample was determined from the diameter by assuming that the samples were cylindrical in shape. Two skinned samples were tested from each heart, and the mean values were used to represent each animal.

Maximal Active Stress

Maximal active force was determined by transferring the sample from the relaxing solution through a washing solution, and into a maximal activating solution ($\text{pCa} = -\log[\text{Ca}^{2+}] = 4.2$). Length was held constant while the total force produced was recorded. Once peak force was reached, the sample was returned to the relaxing solution to deactivate. Maximal active force was calculated as the difference between total force and resting passive force preceding the contraction, as has been described previously (Boldt et al. 2018). Force was normalized to the samples cross sectional area to allow for comparisons of active stress across samples.

Passive Stress

While in the relaxing solution, samples were stretched passively from a resting sarcomere length of $2.2\mu\text{m}$ to an average of $2.42\mu\text{m}$ (10% of the sample's total resting length) at a rate of 5% fibre length/second. Once the sample had been stretched by 10%, length was held constant for 20 seconds to allow for stress relaxation, before being returned to the initial length ($2.2\mu\text{m}$). Peak passive force was taken as the maximum value at the end of the stretch, while steady-state passive force was determined as the mean value of the last second after stress-relaxation was complete and force had reached a plateau as described previously (Boldt et al. 2018). Both passive force values were converted to passive stress by normalizing force by the cross-sectional area calculated from the diameter and assuming cylindrical preparations. The same protocol was then repeated for a stretch to an average sarcomere length of $2.53\mu\text{m}$ (15% stretch from initial fibre length) at the same rate of stretch.

Following the passive stretches, samples were reactivated maximally to ensure the sample did not sustain damage. If the maximum active force decreased by more than 15% from the initial contraction before stretch, the data from that fibre was excluded.

Unloaded Shortening Velocity

Maximal shortening velocity was determined at an initial sarcomere length of $2.2\mu\text{m}$ using the slack test as described by Edman (1979). Briefly, samples were transferred to an activating solution and maximally activated. Once force reached a stable plateau, samples were rapidly shortened (in 2 ms) by 10% (ΔL) of the sample's length. By doing so, samples became slack and force dropped to zero. The time from the onset of the rapid shortening until force redeveloped (Δt) was measured. Samples were then re-lengthened, relaxed, and allowed to rest for five minutes. This rapid shortening test was repeated for ΔL of 11%, 12%, and 13% of the sample's length. The

slope of the linear relationship between ΔL and Δt was used to determine the unloaded (maximal) shortening velocity. Shortening velocity was normalized to the sample length (FL/s).

Calcium Sensitivity

Sensitivity of the muscle to calcium was determined by establishing the force-pCa curve in the range of pCa 7.0-4.2, as described previously (Danieli-Betto et al. 1990). The baseline resting force was measured, before transferring the sample to the first solution (pCa7.0). Once force stabilized, the sample was moved to the next solution containing a higher calcium concentration (pCa6.8). Samples were exposed to solutions of continuously increasing calcium concentrations of pCa6.6, 6.4, 6.2, 6.0, 5.8, 5.4, until reaching pCa4.2 (maximal activating solution). Relative force at each calcium concentration was calculated by dividing the difference between the maximum force at each concentration and the baseline force by the difference between the maximum force produced at pCa4.2 and the baseline force. The force-pCa relationship was calculated by approximating the data using a least-squares regression for the Hill equation in SigmaPlot 13. Calcium sensitivity was obtained and quantitatively compared between samples by determining the pCa that yielded half of the maximal tension (pCa₅₀).

Biochemical Testing

Trabecular muscle myosin heavy chain (MHC) composition was determined using SDS-PAGE gel electrophoresis on 4.5% and 7.5% acrylamide stacking and separating gels, respectively. Trabecular strips which were immediately flash frozen at harvest and stored at -80 °C, were ground into a powder and dissolved in a solubilization buffer (62.5 mM Tris HCl, 10% glycerol, 2% SDS, 5% beta-mercaptoethanol, 0.02% bromophenol blue, pH=6.8) at a

concentration of 1mg/40 μ l. Samples were boiled for three minutes, and immediately spun in a centrifuge at 5000 RPM for 15 minutes at 4 °C.

Solubilized samples were loaded (0.5 μ l/well) into 0.75mm thick acrylamide gels and the gels were run in a Biorad Mini-Protean III unit for 10 hours at a constant voltage of 72V, and 25 hours at a constant current of 1mA/gel. Following electrophoresis, the gels were stained with Coomassie Blue for 60 minutes, then de-stained with a 50% ethanol, 7% acetic acid solution for 5 minutes, and a 5% ethanol, 7% acetic acid solution for at least 60 minutes. After de-staining, the gels were scanned with a Biorad scanner and analyzed with Image J for optical density (OD) to determine the relative composition of myosin heavy chains α (α -MHC) and β (β -MHC). Results will be expressed as the composition of α -MHC relative to total MHC (i.e. OD of α -MHC/(OD of α -MHC + OD of β -MHC)).

Solutions

The relaxing solution contained (in mM): 170 potassium propionate, 2.5 magnesium acetate, 20 MOPS, 5 K2EGTA, 2.5 ATP, and 14.5 creatine phosphate, pH 7.0. The activating solution contained (in mM): 170 potassium propionate, 2.5 magnesium acetate, 10 MOPS, 2.5 ATP, and CaEGTA and K2EGTA mixed at different proportions to obtain various values of pCa, pH 7.0. The washing solution contained (in mM): 185 potassium propionate, 2.5 magnesium acetate, 10 MOPS, and 2.5 ATP, pH 7.0. All solutions contained one tablet of protease inhibitors (Complete; Roche Diagnostics, Quebec, Canada) per 100 ml of solution

Analysis

Statistical significance was determined using Mann-Whitney tests to assess differences between dietary groups. Significance was accepted for $p < 0.05$. Pearson correlations were used to

determine the relationships between body mass and body fat percentage with heart mass, active force, shortening velocity, and calcium sensitivity for all control and obese animals.

Results

Animal Morphology

Body mass increased throughout the 14 weeks for all animals (Figure 1). Around week 5, animals in the high-fat high-sucrose diet group began to increase body mass at a greater rate than the animals on the chow diet. At the endpoint, animals in the high-fat high-sucrose group had significantly larger body mass ($p=0.001$) than animals in the control group. Lean body mass was similar between groups, but animals in the high-fat high-sucrose group had a significantly greater percentage of body fat ($p<0.001$) (Table 1).

Hearts from animals in the high-fat high-sucrose group were significantly heavier than hearts from animals in the control group ($p=0.007$) (Table 1, Figure 2). However, when heart mass was normalized to body mass, lean body mass, or tibia length, the differences in heart mass disappeared (Table 1). Absolute heart mass was significantly correlated to both body mass ($r=0.64$, $p=0.008$) and body fat percentage ($r=0.59$, $p=0.016$) (Table 2).

Mechanical Testing

Active and Passive Stress

There were no differences in the maximal active stress between samples from the two groups (Table 3). Similarly, there were no differences in the passive stresses following stretches of 10% and 15% of the sample's initial length, both at the peak of the stretch and following stress

relaxation (Table 3). There were no significant relationships between active stress and body mass ($r=0.17$, $p=0.535$) or body fat percentage ($r=0.44$, $p=0.087$) (Table 2).

Unloaded Shortening Velocity

Sample traces of the slack test experiments are displayed in Figure 3A. Maximum shortening velocity, determined using the slack test, was significantly higher in samples from the control group animals than those from the high-fat high-sucrose group animals ($p=0.046$) (Figures 3A and 3B). Shortening velocity tended to be related to both body mass ($r=-0.48$, $p=0.062$) and body fat percentage ($r=-0.45$, $p=0.079$) (Table 2).

Calcium Sensitivity

Relative force at each calcium concentration is presented in Figure 4A. Animals in the high-fat high-sucrose group had reduced calcium sensitivity compared to control group animals (Figure 4A). pCa_{50} values were significantly lower for animals in the high-fat high-sucrose group compared to controls ($p=0.048$, Figure 4B). There were no significant relationships between calcium sensitivity and body mass ($r=-0.15$, $p=0.621$) or body fat percentage ($r=-0.41$, $p=0.146$) (Table 2).

Biochemical Testing

Figure 5 indicates the mean α -MHC composition relative to the total MHC composition. The composition of β -MHC relative to total MHC would be one minus the value of α -MHC relative to the total MHC composition. There were no differences in the MHC composition between hearts from animals in the control and the high-fat high-sucrose groups (Figure 5).

Discussion

The purpose of this study was to compare the mechanical properties of cardiac muscle between animals fed a high-fat high-sucrose diet resulting in obesity, and animals fed a chow diet during adolescence and maturation. The main findings were that heart preparations from the high-fat high-sucrose animals had significantly lower unloaded shortening velocities and reduced calcium sensitivities relative to the control group animals. These findings indicate a decrease in cardiac mechanical function in animals from the high-fat high-sucrose group compared to normal weight control animals.

Animal age

In the present study, animals were introduced to the high-fat high-sucrose diet at an age of three weeks, and remained on the diet until seventeen weeks of age. Comparing rat age to human age is challenging. In rats, weaning lasts for three weeks (Sengupta, 2013), the onset of sexual maturity begins around six weeks (Adams and Boice 1983), and adolescent-type behaviours, such as risk taking and social play, begin after the eighth week (Spear 2000). Rats do not reach a true skeletal maturity due to the absence of epiphyseal closure in the long bones (Martin et al. 2003). However, male rats achieve around 90% of epiphyseal closure by 8 months (Martin et al. 2003), corresponding to a slowing of growth (around 7-8 months of age) (Sengupta 2013). Therefore, our rats were exposed to the high-fat high-sucrose diet immediately following weaning and remained on this diet during adolescence, throughout puberty, and into young adulthood.

Animal Morphology

As previously shown using the same diets (Collins et al. 2016, Collins et al. 2017; Rios et al. 2019), animals in the high-fat high-sucrose group reached significantly greater body mass and percentage body fat compared to control group animals, indicating the presence of obesity. The

high-fat high-sucrose diet did not affect lean body mass development but rather, led to greater fat content. Given that body composition data are only available at the endpoint, it is difficult to determine when the onset of obesity occurred. However, given the increased rate of gain in body mass in the high-fat high-sucrose group animals compared to the chow-fed control group animals after five weeks on the diet (Figure 1), we speculate that overweight/obesity was a factor for the majority of the dietary intervention period.

Similar to the findings by Leopoldo et al. (2010), the high-fat high-sucrose group animals had significantly larger hearts than the control group animals in absolute terms, but not when normalized to body mass, lean mass, or tibial length. The difference in absolute heart mass for the high-fat high-sucrose group animals was largely due to two animals that had a substantially larger heart than the other animals in that group (Figure 2). Given the evidence that adulthood obesity can lead to maladaptive ventricular hypertrophy (Alpert et al. 2014; Kasper et al. 1992; Wong and Marwick 2007), future work should utilize more sophisticated morphological measurements of the hearts to assess whether childhood obesity may lead to maladaptive hypertrophy. With these two exceptions, and given no statistical differences when heart mass was normalized to body mass and lean mass, it seems that heart mass in young animals is related to body mass/size.

Mechanical Testing

Active Stress

We did not find differences in maximal active stress between samples from the high-fat high-sucrose and control group hearts. Similarly, there were no correlations between active stress and body fat or mass. Data on cardiac muscle force associated with obesity is limited. However, similar results were obtained by Leopoldo et al. (2010) who compared force production from intact

cardiac fibres obtained from 19-week old rats assigned to either a control or a dietary-induced obesity group. Following 15 weeks of obesity induction, they observed no differences in maximal force production between the two groups. Therefore, it seems that maximal fibre/fascicle stress production in young rats is not compromised by obesity. It needs to be tested if maximal force/function of the entire heart is also not affected by obesity. Specifically, ectopic fat accumulation in ventricular and atrial tissue (Montani et al. 2004) may diminish the percentage of contractile material, thus reducing overall cardiac force and performance. Also, it is not known if maximal force production is affected by obesity in old rats, or in young rats, if the obesity induction occurred over a longer time course (>15 weeks) than in this study. Maximal active force in cardiac tissue has also been shown to remain unchanged following exercise training (Boldt et al. 2018; Chung and Diffie 2012; Diffie and Nagle 2003) and aging (Carnes et al. 2004) in addition to obesity induction (Leopoldo et al. 2010).

Passive Stress

In previous work, dysfunctional increases in cardiac stiffness with obesity have been observed (Carroll et al. 1999). Obesity has been shown to lead to greater passive stiffness and indices of diastolic dysfunction (Pascual et al. 2003; Peterson et al. 2004). Russo et al. (2011) compared left ventricular diastolic function using doppler imaging in normal weight, overweight, and obese individuals. They identified significant differences between diastolic flow velocities in the groups stratified by BMI, indicating worse ventricular diastolic filling with increased body fat. These findings were independent of heart mass and other risk factors (Russo et al. 2011). Similarly, following a 12 week dietary-induced obesity intervention in rabbits, increases in cardiac stiffness were observed using a Langendorff isolated heart preparation (Carroll et al. 1999). The authors

attributed the increase in stiffness to deposition of collagen. However, in this study we did not observe differences in passive force properties.

It may be that while 14 weeks of obesity induction was sufficient to affect whole animal morphology, it was insufficient to affect cardiac stiffness. However, another possible explanation for the absence of differences in passive stiffness may be associated with the young age of the animals, which may have limited the negative effects of obesity on cardiac fibrosis. Work on cardiac compliance has primarily been focused on adult populations (Pascual et al. 2003; Russo et al. 2011; Wong et al. 2004), whereas we evaluated cardiac mechanics following obesity during adolescence and maturation. The increased rate of tissue growth during development may have had a protective effect. Furthermore, adult animals with obesity may have been exposed to a myriad of other comorbidities, such as high blood pressure or insulin resistance, that young animals may not have yet developed (Carroll et al. 1999; Paeratakul et al. 2002). These comorbidities may affect passive force in the heart (Arbab-Zadeh et al. 2004; Borlaug and Kass 2011). However, Sharpe et al. (2006) compared diastolic function in human adolescents (less than 17 years) with obesity and found significant impairment of diastolic function. Therefore, it is difficult to say for certain if a protective effect of age may account for our results.

Another possibility is that the muscle skinning process may have limited our ability to measure changes in passive force. In a similar dietary induction protocol as ours, Leopoldo et al. (2010) compared passive forces in intact fibres. They observed significantly greater passive forces in fibres from obese animals, compared to their controls. It has been determined that the process of skinning muscle fibres leads to a decrease in passive force compared to intact fibres (Kentish, et al. 1986). In previous work (Granzier and Irving 1995), it has been determined that cardiac muscle passive stiffness is governed primarily by titin at lengths less than $1.9\mu\text{m}$, and primarily by

connective tissues of the extra cellular matrix at lengths longer than $1.9\mu\text{m}$. Since we were measuring passive force properties at the end and beyond the physiological cardiac sarcomere length ranges ($1.7\text{-}2.3\mu\text{m}$) (Granzier and Irving 1995; Weiward et al. 2000), passive stiffness in this region of the force-length relationship is expected to be due to connective tissues primarily. Given that connective tissue networks are disturbed during the skinning process (Kentish et al. 1986), it is possible that increases in passive forces due to connective tissue in obese animals may not have been captured in our skinned fibre preparations. Further work on the passive properties of entire hearts in adolescence obesity models is needed to determine if obesity causes increased cardiac stiffness as observed in other preparations.

We did not observe differences in passive force in skinned cardiac fibres between high-fat high-sucrose and control group animals. We speculate that this result is either due to a protection of the heart by young age, or that the skinning process eliminated differences that may have been present in the intact fibres and heart.

Unloaded Shortening Velocity

Ventricular muscular contraction occurs primarily in two phases: isovolumetric contraction (isometric contraction) and ventricular ejection (concentric contraction). During the concentric contraction, the ability of the heart to eject blood depends on the myocardium power, or its ability to perform work at a given rate. A decrease in force or a decrease in shortening velocity would result in decreased myocardial power, and a reduced ability to eject blood. Since there was no difference in maximum isometric force, the isovolumetric phase of the heartbeat cycle would likely be unaffected. However, the reduced shortening velocity in the fibres from the high-fat high-sucrose diet compared to the control group animal hearts indicates a decreased myocardial power,

and an associated decrease in ability to eject blood during the ejection phase of the cardiac cycle. However, it is not known how this result in isolated fibres affects the in-vivo function of the heart.

Previous work has shown a slowing of the contractile speed of the heart with aging (Carnes et al. 2004; Chung and Diffie 2011; Wahr et al. 2000) and an increase in contractile speed with exercise training (Chung and Diffie 2012; Diffie and Chung 2003). Diffie and Chung (2003) compared loaded shortening velocity in skinned rat ventricular fibres between control animals and animals that were treadmill trained for 11 weeks. They observed no differences in maximal isometric force, but an increased shortening velocity in fibres from the exercised hearts at corresponding loads and an associated increase in peak power outputs. They concluded that hearts from treadmill trained animals had a greater work capacity than control hearts because of the increase in shortening speeds. Conversely, Dong et al. (2006) compared shortening speeds in intact ventricular fibres from leptin deficient obese mice. Hearts from these obese animals had slower shortening velocities compared to controls. Therefore, it appears that the high-fat high-sucrose diet used in young rats in our study resulted in changes similar to those observed in aging rats and opposite to those observed in trained rats.

Cardiac muscle expresses two MHC isoforms: α -MHC and β -MHC (Herron, 2002). Shortening velocity is largely dictated by the relative expression of these two MHC isoforms as α -MHC cells have been shown to shorten 75% faster than β -MHC cells (Herron et al. 2001). Thus, a shift towards the α -MHC isoform expression would be expected to lead to increased shortening velocities. Li et al. (2006) compared shortening speeds in intact ventricular fibres following 12 weeks of leptin-deficient obesity induction in rats. They reported lower rates of shortening in hearts from the obese compared to the control group animals. In addition, they observed a shift in MHC isoform expression toward β -MHC in the obese animals. They concluded that the differences in

shortening speeds between the obese and control group animals were associated with the MHC isoform expression. Conversely, Chung and Diffie (2003) observed greater loaded shortening speeds in cardiac myocytes following 12 weeks of treadmill running without differences in MHC isoform expression. They attributed the differences in shortening speeds to increases in myosin light chain (MLC)-1 composition observed in the trained animals. We did not observe differences in MHC expression (Figure 4) and did not test for MLC composition. More work should be done to identify the molecular mechanisms of differences in shortening speeds.

Calcium Sensitivity

Reports from previous studies indicate a reduced responsiveness of myocytes to extracellular calcium in hearts from obese group compared to control group animals (Dong et al. 2006; Li et al. 2006; Relling et al. 2006). The primary focus of these studies was on the processes of calcium delivery into the fibres. Relling et al. (2006) attributed the decreased responsiveness to calcium in myocytes from obese animals to compromised intra-cellular calcium re-sequestering. The results from these past studies suggest that calcium handling and delivery into the cells is impaired in obesity. The removal of cell membranes through chemical skinning, as used in the present study, allows for measurement of the force response of the contractile material of the cells, independent of calcium supply (Hellam and Podolsky 1969). In addition to the impaired distribution of calcium previously reported, we identified that the mechanical machinery of the muscle is less sensitive to calcium in hearts from obese animals relative to normal weight controls.

Adaptation of Ca^{2+} sensitivity has been shown to be related to alterations in the regulatory proteins (such as Troponin C and Troponin T) and MLC isoform expression (Akella et al. 1997; Gulati et al. 1991). For example, Diffie and Nagle (2003) determined calcium sensitivity in exercise trained and sedentary animals using skinned myocytes. They observed greater calcium

sensitivity in the trained compared to the sedentary animals, and reported a correlation ($r=0.72$) between pCa_{50} and the relative expression of MLC-1.

These results indicate that in addition to a decreased calcium supply to the contractile elements (as shown previously (Dong et al. 2006; Li et al. 2006; Relling et al. 2006)), the contractile elements in the skinned fibres are less sensitive to calcium activation. This places the obese heart at a further disadvantage, as it requires a higher activation to produce the same force, and it has impaired calcium delivery. Decreased force output, or increased effort required to produce force, may lead to progressive overworking of the heart, and may be a mechanism for cardiovascular disease later in life.

Future Directions

Future work should evaluate obesity in older rats than the rats used in this study to better understand the effects of obesity acquired during adolescence and during adulthood on the contractile properties and function of the heart. Furthermore, studies should also be conducted with rats on a diet for a longer duration than the 14 weeks of exposure used in our study to evaluate the consequences of obesity over the entire lifespan. Additionally, in-vivo measures, such as pressure-volume measurements, may provide insight into how mechanical adaptations of isolated muscle translate into in-vivo function and should be explored in future work. Finally, exercise has been shown to have effects opposite to those observed with obesity; for example, increasing the maximal shortening velocity (Diffie and Chung 2003; Chung and Diffie 2012) and increasing calcium sensitivity (Diffie et al. 2001; Diffie and Nagle 2003). The potential of reversing or preventing the negative effects of obesity on cardiac function through exercise, or through targeted diet interventions, should be further explored, as it offers a simple and non-invasive way to combat heart failure and disease.

Conclusion

Obesity is a significant independent risk factor for cardiovascular disease (Calle et al. 1999; Kenchaiah et al. 2002). Further, obesity during childhood increases the risk of cardiovascular events later in life, regardless of behaviours adopted during adulthood (Franks et al. 2010; Must et al. 1992). Therefore, it appears that obesity during childhood leads to partly irreversible decrements in cardiac function. However, little is known about how obesity affects the mechanical properties of the heart muscle during development. We observed that obesity in an adolescent rat model resulted in no differences in active or passive force properties, but resulted in significantly slower unloaded shortening velocities and lower calcium sensitivity in isolated skinned cardiac trabecular muscle. We suggest that these two changes observed with obesity may negatively affect cardiac health in adulthood. However, more research is needed to further elucidate the mechanisms for these mechanical differences.

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GM and WH conceived and carried out animal work; KB, VJ, and WH conceived experiments and analyzed data. KB carried out experiments. All authors were involved in writing the paper and had final approval of the submitted and published versions.

Conflicts of Interest

The authors declare no conflicts of interest.

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Tables

	Body Mass (g)	Lean body mass (g)	Body fat (%)	(g)	Heart mass/body mass (mg/g)	Heart mass/lean body mass (mg/g)	Heart mass/tibia length(g/cm)
Control	557±13	468±10	16.1±1.0	1.56±0.03	2.82±0.12	3.37±0.14	0.33±0.01
Obese	671±24*	479±14	28.4±1.2*	1.84±0.10*	2.74±0.13	3.84±0.21	0.38±0.2

Table 1. Descriptive end-point data from animals in the control and the high-fat high-sucrose groups. Data displayed are means±1SEM. * indicates a significant difference from the control group animals with p<0.05.

	Heart Mass	Active Force	Shortening Velocity	pCa
Percentage Body Fat				
r=	0.64	0.44	-0.45	-0.41
p=	0.008*	0.087	0.079	0.146
Body Mass				
r=	0.59	0.17	-0.48	-0.15
p=	0.016*	0.535	0.062	0.621

Table 2. Correlations between body mass and percentage body fat to heart mass, active stress, shortening velocity, and calcium sensitivity (pCa50). * indicates significant correlations with $p < 0.05$

	Maximal Active Stress (kN/m²)	Passive 10% Peak Stress (kN/m²)	Passive 10% Steady State Stress (kN/m²)	Passive 15% Peak Stress (kN/m²)	Passive 15% Steady State Stress (kN/m²)
Control	18.6±1.7	13.1±1.4	9.1±1.0	22.6±2.6	14.8±1.7
Obese	22.8±2.2	15.6±2.4	10.1±1.9	24.5±4.3	16.3±3.1

Table 3. Active and Passive stress data from skinned trabeculae preparations for animals in the control and the high-fat high-sucrose groups. Data displayed are means±1SEM. No significant differences in active and passive stress were obtained between groups.

Figures

Figure 1: Mean $\pm$ SEM values for body mass from each group over the course of the dietary intervention. * indicates a significant differences between groups ($p<0.05$).

Figure 2: Individual values for heart mass stratified by group. Each dot represents the value for each animal.

Figure 3: A: Representative traces from slack tests for both groups. First hashed line indicates the onset of Δt where the fibre is slack and no tension is recorded. The second and third hashed lines mark the redevelopment of tension and indicate the samples have shortened the distance of ΔL . The sample from the control group begins to redevelop tension before the sample from the obese group, reflecting a higher shortening rate in the control. B: Mean $\pm$ SEM values for each group for unloaded shortening velocities. * indicates a significant difference compared to control group ($p<0.05$).

Figure 4: A: Mean relative force and pCa relationships for each group. B: Mean $\pm$ SEM values for each group for pCa50. * indicates a significant difference compared to control group ($p<0.05$).

Figure 5: A: Representative gels from each group indicating the two MHC isoforms expressed in the rat heart (α -MHC and β -MHC). B: Mean $\pm$ SEM values for each group for α -MHC composition relative to total MHC. The composition of β -MHC relative to total MHC would be one minus the value of α -MHC composition relative to total MHC. No significant difference between control and obese group animals was observed.

Figure 1: Mean $\pm$ SEM values for body mass from each group over the course of the dietary intervention. * indicates a significant differences between groups ($p < 0.05$).

177x134mm (300 x 300 DPI)

Figure 2: Individual values for heart mass stratified by group. Each dot represents the value for each animal.

127x82mm (300 x 300 DPI)

Figure 3: A: Representative traces from slack tests for both groups. First hashed line indicates the onset of Δt where the fibre is slack and no tension is recorded. The second and third hashed lines mark the redevelopment of tension and indicate the samples have shortened the distance of ΔL . The sample from the control group begins to redevelop tension before the sample from the obese group, reflecting a higher shortening rate in the control. B: Mean $\pm$ SEM values for each group for unloaded shortening velocities. * indicates a significant difference compared to control group ($p < 0.05$).

152x139mm (300 x 300 DPI)

Figure 4: Individual values for heart mass stratified by group. Each dot represents the value for each animal.

338x139mm (300 x 300 DPI)

Figure 5: A: Representative gels from each group indicating the two MHC isoforms expressed in the rat heart (α -MHC and β -MHC). B: Mean $\pm$ SEM values for each group for α -MHC composition relative to total MHC. The composition of β -MHC relative to total MHC would be one minus the value of α -MHC composition relative to total MHC. No significant difference between control and obese group animals was observed.

203x88mm (300 x 300 DPI)