A High-Fat Diet Increases Adiposity but Maintains Mitochondrial Oxidative Enzymes without Affecting Development of Heart Failure with Pressure Overload

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Running title: Fat Intake, Cardiac Hypertrophy, and Heart Failure.

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ABSTRACT

A high fat diet can increase adiposity, leptin secretion, and plasma fatty acid concentration. In hypertension, this scenario may accelerate cardiac hypertrophy and development of heart failure, but could be protective by activating peroxisome proliferator-activated receptors and expression of mitochondrial oxidative enzymes. We assessed the effects of a high-fat diet on the development of left ventricular hypertrophy, remodeling, contractile dysfunction, and the activity of mitochondrial oxidative enzymes. Mice (n = 10-12/group) underwent transverse aortic constriction (TAC) or sham surgery and were fed either a low-fat diet (10% of energy intake as fat) or a high-fat diet (45% fat) for 6 weeks. The high-fat diet increased adipose tissue mass and plasma leptin and insulin. Left ventricular mass and chamber size were unaffected by diet in sham animals. TAC increased left ventricular mass (~70%), and end systolic and diastolic areas (~100 and ~45%, respectively) to the same extent in both dietary groups. The high-fat diet increased plasma free fatty acid concentration and prevented the decline in the activity of the mitochondrial enzymes medium chain acyl-coenzyme A dehydrogenase (MCAD) and citrate synthase that was observed with TAC animals on a low-fat diet. In conclusion, a high-fat diet did not worsen cardiac hypertrophy or left ventricular chamber enlargement despite increases in fat mass and insulin and leptin concentrations. Further, a high-fat diet preserved MCAD and citrate synthase activities during pressure overload, suggesting that it may help maintain mitochondrial oxidative capacity in failing myocardium.

Key words: fatty acids, heart failure, mitochondria, obesity
INTRODUCTION

Diets with a high relative fat content are commonly used for weight loss and maintenance; however, little is known about the effects of fat intake on the development and progression of heart failure. Patients with hypertension and subsequent left ventricular (LV) hypertrophy frequently develop heart failure (19). Recent observations suggest that this process may be either adversely or favorably affected by dietary fat intake (5, 30). Dietary fat affects cardiac gene expression, structure, metabolism, and contractile function through changes in plasma lipids and hormones (39). Long-chain fatty acid oxidation supplies most of the metabolic fuel for the healthy heart but is reduced in heart failure due to decreased expression and activity of fatty acid oxidation enzymes (39). Genetic rat and mouse models of increased cardiac fatty acid uptake have greater cardiac triglyceride accumulation, LV chamber enlargement, and contractile dysfunction (1). In our models of chronic pressure overload, we have found that an extremely high-fat diet (60% of energy intake from fat) either attenuated (9, 25, 26, 36, 37) or did not affect (4) the development of LV chamber enlargement and heart failure compared to a standard low-fat diet.

Animals consuming a low-fat diet respond to pressure overload with a decrease in the activity of mitochondrial enzymes involved in fatty acid oxidation which may be prevented by high fat intake (3, 6). Long-chain fatty acids activate peroxisome proliferator activated receptor-α (PPARα), which increases the expression of key enzymes of lipid oxidation (15). We observed that a high-fat diet in the absence of obesity prevented the decline in the activity of the fatty acid beta-oxidation enzyme medium chain acyl-coenzyme A dehydrogenase (MCAD) in rat and mouse models of pressure overload-induced LV hypertrophy and heart failure (4, 26). Heart failure decreases mitochondrial oxidative capacity in skeletal muscle (22), and in the absence of
obesity, a high-fat diet increases the activity and expression of mitochondrial enzymes in skeletal muscle of normal rats (13). Thus, a high-fat diet may also improve skeletal muscle mitochondrial oxidative capacity in heart failure.

While these findings suggest that a high-fat diet may be optimal for preventing LV remodeling and myocardial contractile and metabolic dysfunction in hypertension, these data were obtained in the absence of any difference in body mass between dietary treatments. An increase in adipose mass is a major confounding variable when assessing the effects of a high-fat diet on the heart, as it can elevate secretion of leptin from adipocytes exerts pro-inflammatory effects in a variety of tissues (21) and can trigger hypertrophy in isolated myocytes (16). Recent studies in normotensive mice found that long-term consumption of a high-fat diet (45% of energy intake) did not adversely affect cardiac function when energy intake was restricted to prevent an increase in fat mass (10). When high-fat chow was provided ad libitum, an increase in adipose stores ensued with either no evidence for cardiac pathology (42) or moderate cardiac dysfunction (10). Obesity can increase circulating C-reactive protein (CRP) and decrease the anti-inflammatory adipokine adiponectin (7, 8), thereby accelerating cardiac dysfunction and ventricular remodeling (27). Thus, in the setting of chronic hypertension, an increase in fat mass induced by a high-fat diet could worsen the development and progression of heart failure via stimulation of inflammatory processes and leptin-induced cardiac growth. At present, experimental evidence for this concept is lacking.

The purpose of the present study was to determine if an increase in adipose tissue mass induced by a moderately high-fat diet (45% of energy from fat) would accelerate the development of cardiac hypertrophy and heart failure in response to severe pressure overload. This diet was selected because it is similar to the relative fat intake seen in commonly-prescribed
human diets, and because it increases adipose mass and serum leptin in mice (10). We hypothesized that the high-fat diet would increase adipose tissue mass and circulating leptin, insulin, and CRP, decrease plasma adiponectin, and accelerate LV hypertrophy and heart failure under conditions of severe pressure overload. Studies were performed in the well-established mouse model of pressure overload induced by transverse aortic constriction (TAC) (3, 4, 6). Animals were fed either a standard low-fat/high-carbohydrate diet (10% of energy from fat, 70% from starch) or a moderately high-fat diet (45% of energy from fat, 35% from starch). We assessed the effects of diet and TAC on LV mass and chamber size, serum leptin and insulin, the mRNA expression and activity of mitochondrial enzymes, and molecular markers of heart failure (atrial natriuretic factor (ANF) and myosin heavy chain-β (MHC-β)). We also measured markers of inflammation, specifically circulating levels of CRP and adiponectin and cardiac mRNA levels for the inflammatory cytokines tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6). Since heart failure decreases mitochondrial oxidative capacity in skeletal muscle (22) and a high fat diet can up-regulate oxidative capacity (13), we also assessed the effects of TAC and diet on the activity of skeletal muscle mitochondrial enzymes.
METHODS

Study Design. The goal of this study was to assess the effects of a high fat diet that increases body weight and adipose tissue mass on the development of LV hypertrophy and heart failure in mice subjected to severe chronic pressure overload. Previously, we demonstrated that mice subjected to moderate severity TAC (banded using a 26-gauge needle) and fed a cocoa butter-derived high-fat diet (60% fat) comprised of saturated fatty acids (mainly stearate and palmitate) did not become obese and had a similar degree of LV hypertrophy, chamber enlargement and contractile dysfunction as mice fed a standard low-fat lab chow (10% fat) (4). Thus, in the present study, we subjected animals to more severe TAC (27-gauge needle) and fed a lard-based diet (45% fat) high in monounsaturated fatty acids (mainly oleate) in order to induce a greater increase in adipose and body mass (10, 42). One day following TAC or sham surgery, mice were assigned to dietary treatment and maintained for 6 weeks. Each surgical and/or dietary treatment group had 10-12 animals at onset. After 6-weeks of treatment, LV dimensions were assessed by echocardiography, followed 1-3 days later by a terminal surgery to harvest tissue and collect plasma and serum samples. All analyses were performed with the investigators blinded to treatment. This study was conducted according to the Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health publication No. 85-23) and was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland at Baltimore.

Induction of Pressure Overload. Transverse aortic constriction (TAC) was performed on male C57BL/6J mice (aged 10 weeks, 28-32 g) as described previously using a 27-gauge needle (6, 14, 18). The sham procedure was identical without aortic ligation. Animals were maintained on
a reverse 12-hour light/dark cycle.

**Diets.** All diets were custom-formulated by Research Diets, Inc. (New Brunswick, NJ) and were matched for micronutrient content. As a percentage of the total energy, the low-fat diet contained 70% carbohydrate (58% energy from cornstarch and 12% from maltodextrin) and 10% fat (7% lard and 3% soybean oil). The high-fat diet derived 45% energy from fat (42% lard and 3% soybean oil) and 35% from cornstarch. The fat in the chow was comprised mostly of oleate and palmitate (19% and 11% of total energy in the chow, respectively), with relatively little stearate (6%). All diets contained 20% protein from casein + L-cystine. Animals were fed a standard commercial rodent chow (Harlan Teklad 2014, Global 14% Protein Rodent Maintenance Diet) for at least one week prior to surgery. One day following surgery, mice were assigned randomly to either the low-fat or high-fat diet. Food and water were provided *ab libitum* for the remaining 6 weeks of the study.

**Echocardiography.** Echocardiographic measurements of LV size and function were performed under isoflurane anesthesia at 6 weeks post-surgery using a VisualSonics Vevo 770 In Vivo Imaging System (Toronto, Ontario, Canada) as previously described (6, 18). LV end diastolic (EDA) and systolic areas (ESA) were planimetered from long-axis cine loops and used to calculate area of fractional shortening (aFS) using the equation \(aFS = \frac{(EDA-ESA)}{EDA} \times 100\%\). Analyses were performed with the investigator blinded to surgical and dietary treatment.

**Blood Pressure Measurements.** Systolic blood pressure was measured at 3 weeks post-surgery using a Hatteras Instruments SC1000 Dual-Channel Blood Pressure Analysis System (Cary,
NC). Animals were subjected to analysis on three consecutive days, each analysis consisting of a 5-minute preliminary acclimation phase followed by 10 minutes of data recording. Systolic blood pressure was recorded each minute for the 10-minute period. Reported values are the means ± SEM for all values obtained over the 3 consecutive days.

Terminal Surgery. After 6 weeks of treatment, animals were subjected to terminal surgery in the fed state between 3 and 6 hours after initiation of the dark phase. Mice were weighed and anesthetized with 1.5-2.0% isoflurane. The thoracic cavity was opened and the heart was removed, the LV dissected, weighed, freeze-clamped, and stored at -80°C. Pooled blood was collected from the thoracic cavity and separated into serum and plasma. Epididymal, retroperitoneal, and subcutaneous fat was removed and weighed.

Metabolic Measurements. Plasma concentrations of glucose, free fatty acids, triglycerides, insulin, and adiponectin along with serum leptin and tissue triglycerides were measured as previously described (3, 4). Glucose and insulin were measured in blood samples collected after a 12-hour fast as well as in blood collected during the terminal surgery when animals were in the fed state. Plasma C-reactive protein (CRP) was measured using a commercial ELISA kit (ALPCO Diagnostics). The tissue activities of medium chain acyl-coenzyme A dehydrogenase (MCAD), citrate synthase, and glucose 6-phosphate dehydrogenase (G6PDH) were measured spectrophotometrically as previously described (6). Succinate dehydrogenase (SDH) activity was measured according to the assay described by St-Pierre et al. (38).
mRNA Expression Analysis. RNA isolation and cDNA synthesis were performed as described previously (3). The expression of atrial natriuretic factor (Nppa), myosin heavy chain α (Myh6) and β (Myh7), tumor necrosis factor α (TNFα), interleukin-6 (IL-6), MCAD, citrate synthase, NADPH oxidase 4 (NOX4), and peptidylpropyl isomerase a (ppia, internal control) were assessed using the following Applied Biosystems TaqMan® Gene Expression Assays: Mm01255747_g1 (Nppa), Mm00440354_m1 (Myh6), Mm00600555_m1 (Myh7), Mm00443258_m1 (Tnf), Mm99999064_m1 (Il6), Mm00431611_m1 (Acadm), Mm00466043_m1 (CS), Mm00479239_g1 (Nox4), and Mm02342430_g1 (ppia). Semi-quantitative RT-PCR was performed using an Applied Biosystems Prism 7900HT Sequence Detection System with the TaqMan® universal PCR master mix and the following protocol: 2 min. at 50° C, 10 min. at 95° C, 40 cycles at 95° C for 15 sec., and 1 min. at 60° C. Transcript levels were normalized to cyclophilin A (ppia), which did not change with surgery or diet. Results were expressed as fold increase relative to the low-fat–fed sham group.

AMPK Western Blot Analysis. Total and phosphorylated AMPK were assessed as previously described in detail (3, 4, 18). Protein was extracted from frozen cardiac tissue, separated by electrophoresis in 10% SDS-PAGE gels, transferred onto nitrocellulose membranes, and incubated with specific antibodies to total and phosphorylated-AMPK (Thr$^{172}$ of the α subunit) (Cell Signaling Technology, Inc.).

Statistical Analysis. All data are presented as means ± SEM. Two-way analysis of variance (ANOVA) with Bonferroni post hoc adjustment was used to compare dietary groups and surgical treatments. A $p < 0.05$ was accepted as statistically significant.
RESULTS

Survival. Cumulative survival at 6 weeks post-sham surgery in the low-fat– and high-fat–fed sham animals was 90% and 100%, respectively. Survival within low-fat–fed TAC animals was 61.5%, while that of high-fat–fed animals was 78.6%. No significant differences were observed between surgical and/or dietary treatment groups.

Body and Fat Masses. There were no significant differences in body mass at the time of surgery, but the terminal body mass was significantly greater in both the sham and TAC high-fat–fed animals (Table 1). Mice fed the high-fat diet had an increase in the mass of retroperitoneal, epididymal, and subcutaneous fat (Table 1), and a greater sum of fat masses both in absolute terms and when expressed as a percentage of body mass (Figure 1A) compared to the low-fat diet in both sham and TAC groups. TAC decreased the mass of each fat depot and total fat mass in low-fat–fed animals. No differences were observed between sham and TAC in the high-fat–fed animals.

Metabolites and Hormones. Fasting plasma glucose and insulin were unchanged by surgical or dietary treatment (Table 2). As a main effect, high-fat feeding significantly increased insulin concentration in the fed state (Table 2). The insulin level in the low-fat–fed TAC group was significantly less than the high-fat–fed TAC group (Table 2). Plasma glucose concentration in the fed state was unchanged by surgical or dietary treatment.

Serum leptin was increased by high-fat compared to low-fat feeding independent of surgical treatment (Figure 1B) and was positively correlated with the sum of the retroperitoneal, epididymal, and subcutaneous fat masses ($r = 0.96, p < 0.01$). Leptin has been shown to activate
AMP-activated protein kinase (AMPK) (23), which can suppress protein synthesis and hypertrophy in isolated cardiomyocytes (2). Therefore, we assessed AMPK activation in the myocardium (Figure 2). There were no differences in total or phosphorylated AMPK protein levels among groups (Figure 2B). However, the ratio of phosphorylated to total protein was significantly decreased by TAC compared to sham (Figure 2C). High-fat feeding increased plasma free fatty acid concentration as a main effect (Table 2). Within TAC groups, the high-fat diet produced significantly greater free fatty acid concentration compared to the low-fat diet.

Tissue Triglyceride Content. Plasma, cardiac, and liver triglyceride concentrations were unchanged by surgical or dietary treatment (Table 2). Within skeletal muscle, triglyceride concentration was significantly increased by high-fat feeding as a main effect. Low fat-fed TAC animals had significantly lower skeletal muscle triglyceride concentration compared to their respective sham and high-fat–fed TAC animals (Table 2).

Blood Pressure. No differences in systolic blood pressure were observed at 3 weeks regardless of surgical or dietary treatment (Table 1).

Heart Mass. TAC increased the mass of the total heart, left ventricle, and atria in both low-fat– and high-fat–fed animals (Table 1, Figure 3A). Right ventricular mass was unchanged by surgical or dietary treatment (Table 1). There were no significant dietary effects within either the sham or TAC groups.

Left Ventricular Dimensions and Molecular Markers of Heart Failure. TAC increased left ventricular end diastolic and systolic areas compared to sham in both the low- and high-fat–fed
animals (Figure 3B, C). Area of fractional shortening (aFS) was decreased comparably by TAC in low- and high-fat–fed TAC groups compared to sham (Figure 3D). Dietary treatment had no significant effects on LV dimensions. TAC increased the mRNA expression of ANF, MHCβ and NOX4 in low- and high-fat–fed mice compared to sham (Figure 5). No change was observed in MHCα expression regardless of surgical or dietary treatment.

**Metabolic Enzymes.** TAC decreased the activities of MCAD and citrate synthase in the low-fat–fed animals compared to sham, but this effect was prevented in the animals fed the high-fat diet (Figure 4). Furthermore, the MCAD to citrate synthase activity ratio was increased significantly in high-fat– compared to low-fat–fed TAC animals (Table 3), suggesting selective up-regulation of fatty acid oxidation in the high-fat TAC group. Sham animals showed no increase in MCAD or citrate synthase activity with high-fat feeding (Figure 4A, C), consistent with our previous findings (4, 24, 26). Cardiac mRNA expression of MCAD was not affected by surgical or dietary treatment. Citrate synthase mRNA expression was increased by high-fat compared to low-fat feeding as a main effect (Figure 5). TAC also reduced the activity of succinate dehydrogenase (SDH) compared to sham animals regardless of dietary treatment (Table 3). There was a trend for the high-fat diet to prevent this depressed SDH activity compared to low-fat–fed animals, but this effect did not reach statistical significance ($p = 0.067$).

Heart failure upregulates glucose 6-phosphate dehydrogenase, which under pathological conditions may fuel superoxide production by NADPH oxidases, cardiac superoxide levels, and increase oxidative damage (6, 11, 12, 35). The activity of glucose 6-phosphate dehydrogenase was increased by TAC in both the low-fat and the high-fat group compared to sham with no significant effect of dietary treatment (Table 3).
Heart failure decreases mitochondrial oxidative capacity in skeletal muscle in rats and humans (22); however, the effects of TAC-induced heart failure on skeletal muscle mitochondrial oxidative enzymes has not been reported. It was recently observed that a high-fat diet in the absence of obesity increases mitochondrial enzymes in skeletal muscle in rats (13). Therefore, we investigated potential changes in the activity of mitochondrial enzymes in the soleus muscle. TAC significantly decreased the activity of MCAD in both dietary groups. The high-fat diet increased MCAD activity in both sham and TAC groups (Figure 4D). There were no significant differences in skeletal muscle citrate synthase (Figure 4B) or SDH (Table 3) activity among groups. There was no effect of surgery or diet on the MCAD to citrate synthase ratio, suggesting that the high-fat diet did not up-regulate the capacity for fatty acid oxidation in skeletal muscle.

**Inflammatory Markers.** TAC increased mRNA expression of IL-6 in both the low- and high-fat–fed groups compared to sham (Figure 5). However, mRNA levels for TNFα were unaffected by TAC or diet. Plasma CRP and adiponectin were also not different among treatment groups (Table 2).
DISCUSSION

The novel finding of the present study is that there is a similar degree of LV hypertrophy, remodeling, and contractile dysfunction in response to severe pressure overload in mice fed a high-fat diet despite displaying characteristics of the metabolic syndrome (e.g., increased adiposity and elevated circulating leptin, insulin, and free fatty acids) compared to mice fed a standard low-fat diet. In addition, treatment with a high-fat diet prevented one of the hallmarks of LV hypertrophy and heart failure – a decrease in the activity of mitochondrial oxidative enzymes – which may help preserve cardiac energetics and slow deterioration of contractile function under conditions of chronic pressure overload. Taken together, these findings support the concept that a high fat diet per se does not accelerate LV hypertrophy and development of heart failure in chronic hypertension.

Our results are in contrast to our previous work in rats with salt-induced hypertension or aortic banding, which found prevention of LV chamber enlargement and improved survival with a high fat diet (60% of energy intake) compared to a standard low fat diet (9, 25, 26, 36, 37). Previous studies also demonstrated that mice subjected to less severe aortic constriction and fed a 60% fat diet for 16 weeks did not increase adipose mass and had a more modest increase in leptin, and exhibited the same degree of LV hypertrophy and dysfunction as mice on a low-fat diet (4). With both 60% and 45% fat feeding in mice, however, plasma free fatty acid concentration increased and corresponded with preserved activity of MCAD and citrate synthase, consistent with the concept that high lipid intake prevents the decline in mitochondrial oxidative capacity under conditions of cardiac hypertrophy and pressure overload.

The effects of a high intake of dietary on the heart during pressure overload are dependent upon the composition of dietary fat, severity of aortic constriction and resulting hypertrophy and
contractile dysfunction, length of feeding, and age of the animals at study onset. In our previous study, we used a high-fat diet derived from cocoa butter, which is high in stearate (39% of total energy in the chow (24)), a saturated fatty acid typically not consumed in large quantities. In the present study, the fat source was lard, and the fat in the chow was comprised mostly of palmitate and oleate (11% and 19% of total energy in the chow, respectively), with relatively little stearate (6%). Aortic constriction in our first study was induced by a 26-gauge needle which, after 16 weeks, yielded a 36% increase in heart mass and a 15% reduction in ejection fraction compared to sham animals. The present study incorporated more severe constriction with a 27-gauge needle, eliciting a 70% increase in total heart mass and a 28% reduction in ejection fraction. Feeding was administered to 10-week-old animals compared to 6-week-old animals previously, with a reduction in the length of feeding from 16 to 6 weeks. The myocardial MCAD to citrate synthase ratio was decreased by TAC in our previous study (4), but in the current investigation with more severe pressure overload we saw a decrease in both MCAD and citrate synthase activities on the low-fat diet. As before, this effect was which was prevented by high-fat feeding (4). Moreover, we observed an increase in the MCAD to citrate synthase ratio in TAC animals on the fat diet compared to the low-fat fed TAC group, suggesting that a high fat diet has a greater effect on the capacity for fatty acid oxidation than on the capacity for citric acid cycle activity under conditions of pressure overload.

The results of the present investigation and our studies in rats (9, 25, 26, 36, 37) and mice (4) contrast sharply with the recent report by Raher et al. (30), which showed that feeding a high-fat diet to C57BL/6J mice with TAC increased LV mass and chamber volume, contractile dysfunction, and mortality compared to TAC mice on a low-fat diet. As in the present study, Raher et al. banded using a 27-gauge needle; however, they used a diet comprised of 60% of
energy from fat (mainly lard) and studied the animals for 28 days post-surgery. They did not observe an increase in body mass but found elevated insulin and glucose levels with impaired glucose tolerance. These differences suggest that the response to a 60% fat diet comprised of lard may trigger a “cardiometabolic syndrome” that is more severe than what is achieved with a 45% fat diet. In our previous study in TAC mice with a 60% fat diet comprised mainly of saturated fat from cocoa butter, we reported similar hypertrophy and LV dysfunction after TAC compared to a low fat diet. Raher et al. attributed the contrasting results of the two studies to differences in the fatty acid profile between lard and cocoa butter (30). However, our results presented herein suggest that the differences may be due to an extremely high intake of lard. Furthermore, we initiated the high-fat diet immediately after surgery in ten-week-old animals, while Raher et al. fed the high-fat diet to seven-week-old mice for nine days prior to surgery, perhaps resulting in a more adverse cardiac response to TAC than with our protocol.

Dietary fat intake can affect fat deposition and adipose endocrine function. In the present study mice fed the high-fat diet had significantly greater fat mass, which correlated strongly with serum leptin concentration. Studies in human and rodent cardiomyocytes show that leptin can directly stimulate hypertrophy and increase reactive oxygen species generation (16, 20, 31, 32, 41). On the other hand, complete leptin deficiency is associated with myocardial lipid accumulation and cardiac pathology (40). Clearly leptin can exert both cardioprotective and harmful effects on the heart, as has been extensively reviewed (1, 16, 17, 29, 33, 34). The impact of elevated leptin on the heart likely depends on the duration exposure, the degree of hyperleptinemia, and any concomitant changes in related hormones (e.g. insulin and adiponectin). Nonetheless, in the current study, we demonstrated that high-fat feeding significantly increases whole-body adiposity and circulating leptin without deleterious effects on
the heart. We did not observe a decrease in serum adiponectin concentration, consistent with the previous observation that there are not lower adiponectin levels until 20 weeks on a high-fat diet (28). This suggests that the increase in adipose mass was insufficient to suppress adiponectin secretion. Additional studies are required with a more prolonged and severe diet-induced increase in adipose mass to clarify the role of alterations in adipokines in the development and progression of heart failure.

Markers of inflammation were measured to determine if dietary macronutrient composition has an effect on the inflammatory response to aortic banding. There was no change in plasma CRP (Table 1) or mRNA expression of TNF-α. Expression of IL-6 was up-regulated with TAC compared to sham, suggesting that inflammatory mediators are regulated differentially in the development of hypertrophy and contractile dysfunction. A dietary effect on IL-6 expression was not observed, suggesting that cornstarch or the fatty acids within the lard-based Western diet were not sufficient to modify inflammation.

In conclusion, this study demonstrates that a high-fat diet that increases adiposity and serum leptin does not worsen cardiac hypertrophy or contractile dysfunction in response to pressure overload. Further, treatment with a high-fat diet preserved the activities of mitochondrial enzymes compared to low-fat–fed animals, implying a beneficial effect of high-fat diets on the stressed myocardium. Further studies are needed to determine if chronic consumption of high fat can maintain normal mitochondrial function and slow the progression of heart failure.
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REFERENCES


18. **Lei B, Chess DJ, Keung W, O'Shea KM, Lopaschuk GD, Stanley WC.** Transient activation of P38 MAP kinase and up-regulation of Pim-1 kinase in cardiac hypertrophy despite no activation of AMPK. *J Mol Cell Cardiol* 45: 404-410, 2008.


Table 1. Gravimetric data obtained during the terminal surgery following 6 weeks of dietary treatment.

<table>
<thead>
<tr>
<th></th>
<th>LOW FAT</th>
<th>HIGH FAT</th>
<th>TAC vs. Sham</th>
<th>Low vs. High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>TAC</td>
<td>Sham</td>
<td>TAC</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td><strong>Terminal Body Mass (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Fat</td>
<td>36.4 ± 2.3</td>
<td>30.5 ± 1.8 †</td>
<td>40.1 ± 2.4</td>
<td>36.2 ± 2.5 †</td>
</tr>
<tr>
<td>High Fat</td>
<td>952 ± 258</td>
<td>444 ± 110</td>
<td>1539 ± 263</td>
<td>1397 ± 314</td>
</tr>
<tr>
<td><strong>Epididymal fat mass (mg)</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Low Fat</td>
<td>311 ± 79</td>
<td>145 ± 44</td>
<td>592 ± 98</td>
<td>516 ± 116</td>
</tr>
<tr>
<td>High Fat</td>
<td>481 ± 83</td>
<td>269 ± 63</td>
<td>810 ± 159</td>
<td>710 ± 173</td>
</tr>
<tr>
<td><strong>Subcutaneous fat mass (mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Fat</td>
<td>1744 ± 411</td>
<td>858 ± 215 †</td>
<td>2940 ± 503</td>
<td>2623 ± 595 †</td>
</tr>
<tr>
<td>High Fat</td>
<td>116 ± 5</td>
<td>195 ± 7</td>
<td>118 ± 5</td>
<td>185 ± 16</td>
</tr>
<tr>
<td><strong>LV mass (mg)</strong></td>
<td>19.9 ± 0.2</td>
<td>19.4 ± 0.3</td>
<td>20.2 ± 0.1</td>
<td>19.7 ± 0.2</td>
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<td><strong>RV mass/tibial length (mg/mm)</strong></td>
<td></td>
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<tr>
<td>Low Fat</td>
<td>1.44 ± 0.13</td>
<td>1.62 ± 0.16</td>
<td>1.54 ± 0.08</td>
<td>1.86 ± 0.20</td>
</tr>
<tr>
<td>High Fat</td>
<td>0.52 ± 0.06</td>
<td>1.56 ± 0.39</td>
<td>0.52 ± 0.05</td>
<td>1.17 ± 0.25</td>
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<tr>
<td><strong>Total atrial mass/tibial length (mg/mm)</strong></td>
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<td></td>
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<tr>
<td>Low Fat</td>
<td>7.81 ± 0.40</td>
<td>13.22 ± 0.81</td>
<td>7.92 ± 0.31</td>
<td>12.41 ± 1.19</td>
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<tr>
<td>High Fat</td>
<td>110 ± 2</td>
<td>90 ± 9</td>
<td>109 ± 2</td>
<td>107 ± 6</td>
</tr>
</tbody>
</table>

TAC, transverse aortic constriction. Data are means ± SEM. The TAC vs. Sham column represents significant main effects of surgery. The Low vs. High Fat column represents significant main effects of diet. *\( p < 0.05 \) compared to respective sham. †\( p < 0.05 \) compared to low-fat–fed TAC group.
**Table 2. Metabolite and hormone concentrations after 6 weeks of dietary treatment.**

<table>
<thead>
<tr>
<th>Metabolite or Hormone</th>
<th>LOW FAT (Sham)</th>
<th>LOW FAT (TAC)</th>
<th>HIGH FAT (Sham)</th>
<th>HIGH FAT (TAC)</th>
<th>TAC vs. Sham</th>
<th>Low vs. High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 8</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mM) (fasting)</td>
<td>4.34 ± 0.34</td>
<td>3.59 ± 0.68</td>
<td>4.35 ± 0.20</td>
<td>5.25 ± 0.37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Plasma glucose (mM) (fed)</td>
<td>5.24 ± 0.22</td>
<td>5.82 ± 0.35</td>
<td>5.11 ± 0.17</td>
<td>5.41 ± 0.66</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Plasma free fatty acids (mM)</td>
<td>0.24 ± 0.05</td>
<td>0.22 ± 0.02</td>
<td>0.32 ± 0.02</td>
<td>0.34 ± 0.02†</td>
<td>-</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/mL)</td>
<td>70.1 ± 17.1</td>
<td>74.3 ± 13.7</td>
<td>59.0 ± 6.2</td>
<td>50.0 ± 5.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cardiac triglyceride (μmol·(mg ww)⁻¹)</td>
<td>4.08 ± 0.31</td>
<td>3.34 ± 0.62</td>
<td>4.11 ± 0.40</td>
<td>3.28 ± 0.32</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle triglyceride (μmol·(mg ww)⁻¹)</td>
<td>21.8 ± 2.4</td>
<td>11.1 ± 1.8*</td>
<td>29.3 ± 2.4</td>
<td>31.0 ± 3.6†</td>
<td>-</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Liver triglyceride (μmol·(mg ww)⁻¹)</td>
<td>11.1 ± 2.1</td>
<td>6.34 ± 1.57</td>
<td>10.9 ± 1.9</td>
<td>14.0 ± 3.1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Plasma adiponectin (μg/mL)</td>
<td>1.81 ± 0.19</td>
<td>1.93 ± 0.13</td>
<td>1.85 ± 0.13</td>
<td>2.03 ± 0.21</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Plasma Insulin (fasting)</td>
<td>0.18 ± 0.05</td>
<td>0.09 ± 0.03</td>
<td>0.24 ± 0.07</td>
<td>0.25 ± 0.07</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Plasma Insulin (fed)</td>
<td>0.13 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.19 ± 0.05</td>
<td>0.25 ± 0.05†</td>
<td>-</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Plasma C-reactive protein (ng/mL)</td>
<td>11.5 ± 0.6</td>
<td>11.6 ± 0.3</td>
<td>11.7 ± 0.3</td>
<td>10.9 ± 0.3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**TAC, transverse aortic constriction.** Data are means ± SEM. The TAC vs. Sham column represents significant main effects of surgery. The Low vs. High Fat column represents significant main effects of diet. *p < 0.05 compared to respective sham. †p < 0.05 compared to low-fat–fed TAC group.
Table 3. *Enzyme activities in the left ventricular myocardium and soleus after 6 weeks of dietary treatment.*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>LOW FAT</th>
<th>HIGH FAT</th>
<th>Sham vs. TAC</th>
<th>Low vs. High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>TAC</td>
<td>Sham</td>
<td>TAC</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>Myocardial G6PDH</td>
<td>0.203 ± 0.015</td>
<td>0.247 ± 0.023</td>
<td>0.161 ± 0.010</td>
<td>0.225 ± 0.014</td>
</tr>
<tr>
<td>(μmol·gww⁻¹·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial MCAD:CS</td>
<td>0.171 ± 0.006</td>
<td>0.167 ± 0.003</td>
<td>0.169 ± 0.003</td>
<td>0.182 ± 0.005†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial SDH</td>
<td>3.20 ± 0.07</td>
<td>2.03 ± 0.14</td>
<td>3.09 ± 0.23</td>
<td>2.40 ± 0.18</td>
</tr>
<tr>
<td>(μmol·gww⁻¹·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus MCAD:CS</td>
<td>0.036 ± 0.001</td>
<td>0.034 ± 0.002</td>
<td>0.039 ± 0.001</td>
<td>0.037 ± 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus SDH</td>
<td>1.42 ± 0.12</td>
<td>1.37 ± 0.11</td>
<td>1.44 ± 0.09</td>
<td>1.50 ± 0.06</td>
</tr>
</tbody>
</table>

TAC, transverse aortic constriction; G6PDH, glucose 6-phosphate dehydrogenase; MCAD, medium chain acyl-coenzyme A dehydrogenase; CS, citrate synthase. Data are means ± SEM. The TAC vs. Sham column represents significant main effects of surgery. The Low vs. High Fat column represents significant main effects of diet. †p < 0.05 compared to low-fat–fed TAC group.
Figure Legends.

**Figure 1.** The sum of the retroperitoneal, epididymal, and subcutaneous fat masses expressed as a percentage of body mass (A) and serum leptin concentration (B). Data are means ± SEM. *p < 0.05 compared to sham animals on the same diet. Brackets indicate a significant main effect of high-fat compared to low-fat feeding.

**Figure 2.** Representative blots and quantified densitometry for phosphorylated (A) and total (B) AMPK protein levels in left ventricular tissue after 6 weeks of treatment. Labels above gel lanes designate the following: LFS, low-fat sham; LFT, low-fat TAC; HFS, high-fat sham; HFT, high-fat TAC. (C) Phosphorylation relative to total protein. Differences and interactions between surgical and dietary groups were assessed by 2-way ANOVA. Data are means ± SEM. *p < 0.05 compared to respective sham.

**Figure 3.** LV mass (A), end diastolic area (EDA) (B), end systolic area (ESA) (C) and area of fractional shortening (aFS) (D) 6 weeks after surgery and initiation of dietary treatment. Data are means ± SEM. *p < 0.05 compared to respective sham.

**Figure 4.** Activity of the mitochondrial enzymes citrate synthase (A, B) and medium-chain acyl-CoA dehydrogenase (MCAD) (C, D) in myocardium (*left panels*) and in skeletal muscle (*right panels*). Data are means ± SEM. *p < 0.05 compared to respective sham. †p < 0.05 compared to low-fat–fed TAC group. Brackets indicate a significant main effect of high-fat compared to low-fat feeding.
**Figure 5.** mRNA expression of selected genes measured by semi-quantitative RT-PCR. Expression was normalized to cyclophilin A, an internal control, which did not change with surgical or dietary treatment, and the mean of the low-fat–fed sham group (dashed line). *(Left)* Atrial natriuretic factor (ANF), myosin heavy chain α (MHCα), and myosin heavy chain β (MHCβ); *(Right)* tumor necrosis factor α (TNFα), interleukin-6 (IL-6), medium-chain acyl-CoA dehydrogenase (MCAD), and citrate synthase (CS). Data are means ± SEM. *p < 0.05 compared to respective sham. Brackets indicate a significant main effect of high-fat compared to low-fat feeding.
Figure 1.

A. Fat Mass (% Body Mass)

- Sham
- TAC

B. Serum Leptin (ng/mL)

- LOW FAT
- HIGH FAT

* p < 0.05

* p < 0.01
Figure 2.

A

B

C

Phospho-AMPK (AU)

Sham  TAC

LOW FAT  HIGH FAT

Total AMPK (AU)

LOW FAT  HIGH FAT

Phospho-/Total AMPK (AU)

LOW FAT  HIGH FAT

*
Figure 3.

(A) LV:Tibia (mg:mm) for Sham and TAC groups in LOW and HIGH FAT conditions.

(B) EDA (mm²) for Sham and TAC groups in LOW and HIGH FAT conditions.

(C) ESA (mm²) for Sham and TAC groups in LOW and HIGH FAT conditions.

(D) aFS (%) for Sham and TAC groups in LOW and HIGH FAT conditions.
Figure 4.

A. 

**Myocardium**

Citrate Synthase Activity (μmol·gww⁻¹·min⁻¹)

- LOW FAT
- HIGH FAT

B. 

**Skeletal Muscle**

Citrate Synthase Activity (μmol·gww⁻¹·min⁻¹)

- LOW FAT
- HIGH FAT

C. 

Sham  TAC

MCAD Activity (μmol·gww⁻¹·min⁻¹)

- LOW FAT
- HIGH FAT

D. 

- LOW FAT
- HIGH FAT

P < 0.05
Figure 5.

The figure shows a bar graph comparing mRNA expression for various genes across different groups. The x-axis represents different genes: ANF, MHCα, MHCβ, IL-6, TNFα, MCAD, and CS. The y-axis represents mRNA expression (fold change relative to starch sham). The groups are grouped as follows:

- Low Fat Sham
- Low Fat TAC
- High Fat Sham
- High Fat TAC

- **ANF**
  - Low Fat Sham: Significantly higher than other groups
  - Low Fat TAC: Significantly higher than other groups
  - High Fat Sham: Not significantly different
  - High Fat TAC: Significantly higher than other groups

- **MHCα**
  - Low Fat Sham: Not significantly different
  - Low Fat TAC: Significantly higher than other groups
  - High Fat Sham: Significantly higher than other groups
  - High Fat TAC: Significantly higher than other groups

- **MHCβ**
  - Low Fat Sham: Not significantly different
  - Low Fat TAC: Significant increase
  - High Fat Sham: Not significantly different
  - High Fat TAC: Significant increase

- **IL-6**
  - Low Fat Sham: Not significantly different
  - Low Fat TAC: Significant decrease
  - High Fat Sham: Not significantly different
  - High Fat TAC: Significant decrease

- **TNFα**
  - Low Fat Sham: Not significantly different
  - Low Fat TAC: Not significantly different
  - High Fat Sham: Not significantly different
  - High Fat TAC: Not significantly different

- **MCAD**
  - Low Fat Sham: Not significantly different
  - Low Fat TAC: Not significantly different
  - High Fat Sham: Not significantly different
  - High Fat TAC: Not significantly different

- **CS**
  - Low Fat Sham: Not significantly different
  - Low Fat TAC: Not significantly different
  - High Fat Sham: Not significantly different
  - High Fat TAC: Not significantly different

Significance levels are indicated with asterisks (*), with p < 0.05.