Deleterious effects of sugar and protective effects of starch on cardiac remodeling, contractile dysfunction, and mortality in response to pressure overload

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Deleterious effects of sugar and protective effects of starch on cardiac remodeling, contractile dysfunction, and mortality in response to pressure overload. Am J Physiol Heart Circ Physiol 293: H1853–H1860, 2007. First published July 6, 2007; doi:10.1152/ajpheart.00544.2007.—Little is known about the effects of the composition of dietary carbohydrate on the development of left ventricular (LV) hypertrophy (LVH) and heart failure (HF) under conditions of pressure overload. The objective of this study was to determine the effect of carbohydrate composition on LVH, LV function, and mortality in a mouse model of chronic pressure overload. Male C57BL/6J mice of 6 wk of age (n = 14–16 mice/group) underwent transverse aortic constriction (TAC) or sham surgery and were fed either standard chow (STD; 32% corn starch, 35% sucrose, 3% maltodextrin, and 10% fat expressed as a percent of the total energy), high-starch chow (58% corn starch, 12% maltodextrin, and 10% fat), or high-fructose chow (9% corn starch, 61% fructose, and 10% fat). After 16 wk of treatment, mice with TAC fed the STD or high-fructose diets exhibited increased LV mass, larger end-diastolic and end-systolic diameters, and decreased ejection fraction compared with sham. The high-starch diet, in contrast, prevented changes in LV dimensions and contractile function. Cardiac mRNA for myosin heavy chain-β was increased dramatically in the fructose-fed banded animals, as was mortality (54% compared with 8% and 29% in the starch and STD banded groups, respectively). In conclusion, a diet high in simple sugar was deleterious, resulting in the highest mortality and expression of molecular markers of cardiac dysfunction in TAC animals compared with sham, whereas a high-starch diet blunted mortality, increases in cardiac mass, and contractile dysfunction.

Glycemic index; ejection fraction; heart failure

Chronic arterial pressure overload is a major cause of left ventricular (LV) hypertrophy (LVH), which frequently progresses to heart failure (HF). Even with modern pharmacological treatment, many patients with hypertension still develop LVH and HF (44). Thus, novel therapies are needed to prevent LVH and/or the development of HF, especially those that act independently of the neurohormonal axis. Nutritional approaches are particularly attractive because they could work additively with medicinal therapies while not exerting negative hemodynamic effects (37).

While a high-carbohydrate/low-fat diet is recommended for the prevention of heart disease (21), a recent epidemiological study (12) found a twofold increase in the risk of coronary heart disease in women consuming a diet of high glycemic index (i.e., one containing foods rich in sugars and rapidly digested polysaccharides) compared with a diet low in sugar. Diets with a high glycemic load influence the development of obesity and metabolic syndrome, which have become an epidemic in this country and are positively associated with cardiovascular disease risk (33). The occurrence of such risk factors is reduced by diets of a lower glycemic load or those rich in complex carbohydrates (23, 36). Thus, current guidelines suggest that dietary carbohydrate should be comprised mainly of complex carbohydrates (starches) rather than sugars (21).

High-carbohydrate composition may be associated with LVH due to activation of the insulin signaling pathway in the heart (4, 40). Clinical studies (34, 39, 43) have found that hypertensive patients with a high plasma insulin in the fed state have a greater occurrence of LVH, suggesting that elevated insulin may trigger pathological cardiac growth when the heart is subjected to chronic pressure overload. Recently, we (38) observed that feeding a high-carbohydrate diet comprised mainly of fructose to hypertensive Dahl salt-sensitive rats impaired LV function and increased mortality by 85% over a 13-wk period compared with a standard laboratory chow. Studies (4, 40) in transgenic mice have also suggested that the activation of insulin signaling can lead to LVH and cardiac dysfunction. The main effector of insulin signaling is the serine/threonine kinase Akt, whose overexpression results in increased protein synthesis (40), inhibited protein breakdown (27, 41), and subsequently massive LVH (22). Insulin also affects the activity of AMP-activated protein kinase (AMPK), which upregulates glucose and fatty acid utilization during periods of low energy (1, 19). The effects of diet on Akt and AMPK expression and activation in mediating structural and metabolic remodeling of the heart during chronic pressure overload are unclear.

Nuclear receptor signaling is also affected by dietary macronutrients. A recent study (10) in transgenic mouse models of metabolic syndrome has suggested that mitochondrial biogenesis is stimulated by increased lipid availability and activation of peroxisome proliferator-activated receptor-α (PPAR-α) and its coactivators. PPAR-α, which is suppressed in severe LVH and HF, is activated by long-chain fatty acids and regulates the expression of proteins involved in fatty acid metabolism (42). Dietary fat and carbohydrate intake alters the development of LVH and the progression toward HF (30, 38); thus, sugar consumption may accelerate the deactivation of PPAR-α, resulting in impaired cardiac fatty acid metabolism and mitochondrial function under conditions of pressure overload.

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This study evaluated the effect of carbohydrate composition on the development of structural, functional, and metabolic abnormalities in response to pressure overload. We hypothesized that a high-sugar diet would exacerbate cardiac remodeling and dysfunction and increase mortality in mice with aortic constriction. A top-down approach was taken, with the effects of carbohydrate composition assessed by mortality, echocardiographic measurements of LV chamber dimensions and function, and quantification of hormones and metabolites in blood, activity of key metabolic enzymes, gene expression, and hypertrophic signaling pathways. The activation of PPAR-α was assessed through measurements of transcript levels of established PPAR-α target genes and the activity of the fatty acid β-oxidation enzyme medium-chain acyl-CoA dehydrogenase (MCAD).

**METHODS**

**Induction of pressure overload in C57BL/6J mice.** Transverse aortic constriction (TAC) was performed on male C57BL/6J mice at 6 wk of age as previously described (13) using a 26-gauge needle to induce clinically relevant hypertrophy. The sham procedure was identical without aortic ligation. This study was conducted according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23) and was approved by the Institutional Animal Care and Use Committee of Case Western Reserve University. Animals were maintained on a 12:12-h light-dark cycle.

**Diets.** All diets were custom formulated by Research Diets (New Brunswick, NJ). The macronutrient composition of the diets is shown in Table 1. Animals were fed institutionally available laboratory chow (Harlan Teklad 2014, Global 14% Protein Rodent Maintenance Diet) for 1 wk. Following surgery, animals were randomly assigned to either the high-starch (starch), standard (STD), or high-fructose (fructose) diet for the remainder of the study.

**Echocardiography.** LV dimensions and function were measured at 5 and 16 wk postsurgery as previously described (28, 29) using isoflurane anesthesia. Relative wall thickness (RWT) was approximated using the following formula: RWT = (LVWPTd + LVSWTd)/(LVEDD), where LVWPTd is LV posterior wall thickness at end diastole, LVSWTd is LV septal wall thickness at end diastole, and LVEDD is LV end-diastolic diameter. The myocardial performance index (MPI), a reflection of LV systolic and diastolic function whose magnitude is inversely related to myocardial performance, was calculated using the following formula: MPI = (IVCT + IVRT)/(ET), where IVCT and IVRT are isovolumic contraction and relaxation times, respectively, and ET is ejection time. The ejection fraction (EF) was calculated using the following equation: EF = [(LVEDV - LVESV)/(LVEDV)] × 100, where LVEDV and LVESV are LV end-diastolic and end-systolic volumes, respectively.

**Terminal surgery.** Terminal surgeries were performed on fed mice between 3 and 6 h from the completion of the dark phase (18:00) on the light-dark cycle. After 16 wk of treatment, mice were weighed and anesthetized with 1.5–2.0% isoflurane. The thoracic cavity was opened by cutting from the xiphoid cartilage around the ventral portion of the ribs. The heart was removed, weighed, freeze clamped, and stored at −80°C. Pooled blood was collected from the thoracic cavity after heart excision for the analysis of plasma and serum metabolites. Metabolic measurements. Plasma glucose, insulin, free fatty acids, and triglycerides were determined using commercially available kits. Serum leptin and total plasma adiponectin (individual adiponectin isoforms were not assessed) were measured with colorimetric enzyme-linked immunosorbent assays. MCAD and citrate synthase (CS) activities were analyzed from tissue homogenates as previously described (31).

**mRNA expression analysis.** Powdered heart tissue was disrupted and homogenized by being shaken in solution with 5-mm stainless steel beads for 3 min at 30 s−1 (Mixermill 300, Qiagen) followed by RNA isolation (RNeasy Mini Kit, Qiagen). RNA samples were eluted in 50 µl of nuclease-free water and stored at −80°C. For cDNA synthesis, RNA (1 µg) was mixed with 2 µl oligo(dT)16 (Applied Biosystems) and 0.5 µl random primers (Invitrogen) and brought to 15 µl of total volume. The sample/primer mix was heated to 70°C for 10 min and then placed immediately on ice for 2 min followed by the addition of the RT reaction mix containing 5× buffer (5 µl) and 0.1 M DTT (2.5 µl, Superscript II RT, Invitrogen), 10 mM dNTP (1.25 µl, Invitrogen), and RNase inhibitor (0.25 µl, Applied Biosystems). The reaction mix was incubated for 2 min at 42°C, 1 µl reverse transcriptase was added, and the incubation was continued at 42°C for 1 h. The reaction mix was then incubated at 70°C for 10 min and placed on ice for 2 min. The resulting cDNA samples were stored at −20°C. Quantitative RT-PCR (qRT-PCR) was performed using an ABI 7900 and the following protocol: 2 min at 50°C, 10 min at 95°C, and 40 cycles at 95°C for 15 s and 1 min at 60°C. Each reaction was 25 µl consisting of 1.0 µl cDNA sample, 1.25 µl TaqMan Gene Expression Assay, 12.5 µl of 2× TaqMan PCR master mix, and 10.25 µl nuclease-free water. Expressions of atrial natriuretic peptide, myosin heavy chain (MHC)-α, MHC-β, MCAD, CS, PPAR-α, carnitine palmityl transferase I, pyruvate dehydrogenase kinase 4, uncoupling protein 3, and peptidylprolyl isomerase A (internal control) were determined by qRT-PCR using Assays-On-Demand probes (Applied Biosystems). mRNA concentrations were normalized to the fold increase relative to the STD sham group.

**Western blot analysis.** 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 either phosphorylated Akt (Ser473) or phosphorylated AMPK (Thr172 of the α-subunit) (all at 1:1,000, from Cell Signaling Technology). Fluorescence-conjugated secondary antibodies (IRDye 680/800, 1:5,000, LI-COR Bioscience) were used for incubation before the membranes were scanned and analyzed with the Odyssey infrared imaging system (LI-COR Bioscience). Membranes were then stripped (Pierce Restore stripping buffer) and reprobed for total Akt and AMPK (1:1,000, Cell Signaling).

**Statistical analysis.** All analysis was performed with the investigator(s) blinded. Two-way ANOVA with Bonferroni post hoc adjustment was used to compare all dietary groups with and without aortic constriction. Differences in mortality were assessed by a Kaplan-Meier survival analysis.
Meier survival curve with χ²-analysis (SPSS Base 15.0, SPSS, Chicago, IL). All data are presented as means ± SE. *P < 0.05 was accepted as statistically significant.

RESULTS

Survival. All sham-operated mice on the STD and starch diets survived the 16-wk duration of the study. Mortality was observed in mice with TAC on STD and starch diets (29% and 8%, respectively; Fig. 1B). The fructose diet had the most obvious effect on survival, inducing mortality rates in both sham (21%; Fig. 1A) and TAC (54%; Fig. 1B) mice. The mortality observed in TAC mice on the fructose diet was significantly greater than starch diet-fed TAC mice. However, TAC-induced mortality was not different between fructose diet- and STD diet-fed mice compared with sham mice (Fig. 1, A and B).

Body and heart mass. No significant differences in body mass were observed in either sham or TAC animals fed the three diets (Table 2). Average daily food consumption was also not different among groups (data not shown). TAC increased heart weight (normalized to tibia length) by 38.7% in STD diet-fed mice and 25.3% in fructose diet-fed mice compared with sham animals on the same diets (Fig. 2). The starch diet completely prevented the increase in heart weight in mice with TAC. TAC led to a significant reduction in epididymal fat pad mass in fructose diet-fed mice compared with mice on the starch diet (Table 2).

LV dimensions and performance. There were no differences in echocardiographic measurements among dietary and/or surgical groups at 5 wk (data not shown). After 16 wk of treatment, LVEDD and LVESD were increased significantly by TAC in the fructose diet-fed group compared with its respective sham group, but there were no differences between TAC and sham animals in the STD or starch diet groups (Fig. 3, A and B). TAC reduced EF (Fig. 3C) in both STD diet- and fructose diet-fed mice compared with sham mice; however, there was no difference between sham and TAC mice in the starch diet group. The MPI, whose magnitude is inversely related to myocardial performance, was increased significantly in TAC animals on the STD and fructose diets compared with their respective shams; however, there were no differences in MPI between sham and TAC animals on the starch diet (Fig. 3D).

Table 2. Body measurements taken at terminal procedure following 16 wk of dietary treatment

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sham (n = 16)</th>
<th>TAC (n = 14)</th>
<th>Mean ± SE</th>
<th>Sham (n = 10)</th>
<th>TAC (n = 8)</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>28 ± 1</td>
<td>28 ± 1</td>
<td>28 ± 1</td>
<td>27 ± 1</td>
<td>26 ± 1</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>153.4 ± 3.7</td>
<td>160.5 ± 12.9</td>
<td>154.3 ± 5.5</td>
<td>214.0 ± 21.8*</td>
<td>147.2 ± 4.7</td>
<td>184.4 ± 21.6*</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>17.05 ± 0.20</td>
<td>16.97 ± 0.14</td>
<td>16.77 ± 0.21</td>
<td>17.11 ± 0.13</td>
<td>17.12 ± 0.29</td>
<td>17.14 ± 0.14</td>
</tr>
<tr>
<td>Epididymal fat, mg</td>
<td>518.0 ± 36.6</td>
<td>613.1 ± 63.1</td>
<td>541.7 ± 40.6</td>
<td>412.7 ± 71.9</td>
<td>467.1 ± 24.4</td>
<td>383.0 ± 27.2*†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. TAC, transverse aortic constriction. *P < 0.05 vs. sham animals on the same diet; †P < 0.05 vs. TAC animals on the starch diet.

Fig. 1. Percent survival in sham (A) and transverse aortic constriction (TAC; B) mice displayed graphically over the 16-wk duration of the study. Starch, high-starch diet; fructose, high-fructose diet; STD, standard diet. *P < 0.05 compared with TAC mice on the starch diet. Each group contained 14–16 animals at the onset of dietary treatment.

Fig. 2. Terminal heart weight (HW)-to-tibia length ratios of sham (n = 10–16 mice/group) and TAC (n = 8–14 mice/group) animals. Data are means ± SE. *P < 0.05 compared with sham animals on the same diet.
Metabolic parameters. Plasma glucose was unaffected by TAC or dietary treatment. No differences were observed in plasma free fatty acid concentrations among all groups. Plasma triglycerides, however, were increased significantly in both TAC and sham mice on the starch diet compared with all other TAC and sham groups (Table 3). Plasma insulin was similar in sham and TAC animals fed the STD and starch diets but was significantly increased in the fructose-fed TAC group compared with its respective sham (Table 3). Total adiponectin levels were unchanged with dietary manipulation in either sham or TAC mice. Serum leptin was significantly elevated in TAC animals compared with sham animals in the starch diet-fed groups.

Fructose feeding lowered the activity of MCAD in TAC animals relative to sham animals (Fig. 4A). CS activity, a marker of mitochondrial density and oxidative capacity, was reduced in TAC animals consuming STD and fructose diets compared with their respective sham groups (Fig. 4B). The ratio of MCAD activity to CS activity was unaffected by TAC or diet (data not shown), suggesting there was not a preferential downregulation of fatty acid β-oxidation.

mRNA expression. There was a trend toward increases in atrial natriuretic factor mRNA in animals with TAC (Table 4). TAC caused a switch in MHC isoform from α to β, an effect that was enhanced dramatically by fructose diet feeding (Fig. 5) due to a 17-fold increase in MHC-β (Table 4). TAC also reduced the mRNA expression of MCAD and PPAR-α. Within the TAC groups, PPAR-α expression was significantly decreased with the fructose diet compared with the starch diet (Table 4).

Western blot analysis. There were no significant differences in total Akt protein levels or the ratio of phosphorylated Akt (Ser473) to total Akt between any of the treatment groups (Table 5). Similar to total Akt protein levels or the ratio of phosphorylated Akt, there were no significant differences in total AMPK protein levels or the ratio of phosphorylated AMPK (Thr172) to total AMPK (Table 5).

DISCUSSION

The primary findings from this study were that compared with a complex carbohydrate diet, a diet high in sugar increased the mortality and expression of molecular markers of...
findings suggest that dietary sugar composition can promote cardiac remodeling and contractile dysfunction in the presence of chronic pressure overload.

Fructose diet feeding in the TAC mice also decreased the activities of MCAD and CS, marker enzymes for myocardial fatty acid oxidation capacity and citric acid cycle flux, respectively. These decreases in enzyme activity occurred despite no alterations in the mRNA for either MCAD or CS (Table 4). These findings differ from our previous observations showing that MCAD activity and protein expression remained unchanged despite reductions in MCAD gene expression (24) and contrasts with evidence of HF-induced downregulation of fatty acid oxidation genes (8, 32). These conflicting results reinforce the notion that there are frequent disconnections between mRNA and protein levels during LVH and HF (24, 42).

The precise mechanism for increased mortality in the fructose diet-fed TAC group is not known. Previous studies (4, 14, 40) have suggested that enhanced activation of the Akt-insulin pathway could contribute to LVH and contractile dysfunction in transgenic animals and isolated cardiomyocytes. In this study, however, there was no increase in Akt activation in TAC animals on the fructose diet despite a significant elevation in plasma insulin. This discrepancy may be attributed to eating patterns of the mice prior to terminal studies, as it has been shown that Akt activation is highly dependent on nutritional status (40).

There were also no changes observed in the expression and activation of AMPK. To our knowledge, only two studies have measured the effects of cardiac hypertrophy on the activation and expression of AMPK. A study (18) in juvenile pigs with volume overload-induced LVH showed an ~30% decrease in AMPK activity in freeze-clamped myocardial tissue. In contrast, ex vivo perfused hearts from rats subjected to 17–19 wk of ascending aortic banding exhibited an approximately threefold increase in AMPK activity (45). Thus, at this point, the role of this protein in the development of LVH remain unclear.

The expression of PPAR-α mRNA was reduced in the TAC mice fed the fructose diet; however, the expression of PPAR-α-regulated genes (such as MCAD) was unaffected by diet among TAC groups. Nonetheless, the activity of MCAD was suppressed in TAC mice on the fructose diet (Fig. 4). CS activity was also reduced in the fructose diet group, suggesting that fructose diet feeding in the setting of pressure overload

LVH and HF under conditions of pressure overload. The expression of fetal genes, LV contractile dysfunction, and mortality in TAC animals increased as a function of the percentage of calories derived from sugar (Figs. 1–5). These

Table 4. *mRNA expression of cardiac genes determined by quantitative RT-PCR*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Product</th>
<th>Sham (n = 10)</th>
<th>TAC (n = 8)</th>
<th>Sham (n = 9)</th>
<th>TAC (n = 8)</th>
<th>Sham (n = 8)</th>
<th>TAC (n = 7)</th>
<th>P Values (TAC vs. sham)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nppa</em></td>
<td>ANF</td>
<td>0.65 ± 0.15</td>
<td>1.01 ± 0.23</td>
<td>1.00 ± 0.23</td>
<td>1.19 ± 0.17</td>
<td>1.27 ± 0.31</td>
<td>1.50 ± 0.27</td>
<td>0.069</td>
</tr>
<tr>
<td><em>Myh6</em></td>
<td>MHC-α</td>
<td>1.08 ± 0.13</td>
<td>1.00 ± 0.05</td>
<td>1.00 ± 0.09</td>
<td>0.91 ± 0.18</td>
<td>1.03 ± 0.11</td>
<td>0.76 ± 0.16</td>
<td>NS</td>
</tr>
<tr>
<td><em>Myh7</em></td>
<td>MHC-β</td>
<td>0.91 ± 0.14</td>
<td>1.60 ± 0.18</td>
<td>1.00 ± 0.17</td>
<td>2.72 ± 0.38</td>
<td>1.55 ± 0.28</td>
<td>16.59 ± 3.17†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Acadm</em></td>
<td>MCAD</td>
<td>1.06 ± 0.05</td>
<td>0.86 ± 0.07</td>
<td>1.00 ± 0.07</td>
<td>0.90 ± 0.09</td>
<td>0.93 ± 0.09</td>
<td>0.75 ± 0.07</td>
<td>0.015</td>
</tr>
<tr>
<td><em>Cs</em></td>
<td>CS</td>
<td>1.23 ± 0.17</td>
<td>0.97 ± 0.10</td>
<td>1.00 ± 0.13</td>
<td>0.87 ± 0.13</td>
<td>1.04 ± 0.15</td>
<td>0.78 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td><em>Ppara</em></td>
<td>PPAR-α</td>
<td>1.18 ± 0.14</td>
<td>1.00 ± 0.11</td>
<td>1.00 ± 0.13</td>
<td>0.78 ± 0.14</td>
<td>0.94 ± 0.13</td>
<td>0.64 ± 0.13</td>
<td>0.035</td>
</tr>
<tr>
<td><em>Cpt1b</em></td>
<td>CPT-Iβ</td>
<td>1.06 ± 0.13</td>
<td>0.87 ± 0.05</td>
<td>1.00 ± 0.08</td>
<td>0.86 ± 0.07</td>
<td>1.00 ± 0.09</td>
<td>0.91 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td><em>Pdk4</em></td>
<td>PDK4</td>
<td>0.72 ± 0.13</td>
<td>0.67 ± 0.11</td>
<td>1.00 ± 0.20</td>
<td>0.84 ± 0.14</td>
<td>0.78 ± 0.13</td>
<td>0.46 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td><em>Ucp3</em></td>
<td>UCP3</td>
<td>1.21 ± 0.33</td>
<td>1.03 ± 0.19</td>
<td>1.00 ± 0.17</td>
<td>1.05 ± 0.23</td>
<td>1.29 ± 0.48</td>
<td>1.15 ± 0.41</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE of fold changes in gene expression relative to the STD diet-fed sham group; n, no. of animals. ANF, atrial natriuretic factor; MHC, myosin heavy chain; MCAD, medium-chain acyl-CoA dehydrogenase; CS, citrate synthase; PPAR-α, peroxisome proliferator-activated receptor-α; CPT-Iβ, carnitine palmitoyl transferase-Iβ; PDK4, pyruvate dehydrogenase kinase 4; UCP3, uncoupling protein 3; NS, not significant. *P < 0.05 vs. sham animals on the same diet; †P < 0.05 vs. TAC animals on STD and starch diets; ‡P < 0.05 vs. TAC animals on the starch diet.
exacerbated the impairment in mitochondrial oxidative metabolism.

The effects of fructose diet feeding may be mediated by the development of metabolic syndrome (insulin resistance, hyperglycemia, dyslipidemia, hypertension, and/or obesity). The consumption of dietary fructose, particularly in more developed countries in which many foods are enriched with high-fructose corn syrup, has been suggested to contribute to the development of obesity and its corresponding metabolic abnormalities (3, 5, 11, 16, 25). Fructose diet feeding is a well-established model of metabolic syndrome, as it leads to hypertension, hypertriglyceridemia, and insulin resistance in rats (15). Mild insulin resistance was suggested in fructose diet-fed TAC animals as they displayed significantly higher plasma insulin compared with starch diet- and STD diet-fed TAC animals and also exhibited the highest glycemia (Table 3). Secondary to this modest insulin resistance was impaired Akt activation, which has been shown previously (9).

It is also possible that the fructose diet increased blood pressure in TAC mice; thus, a limitation of the present study is the lack of blood pressure data. Studies (20, 26) have shown mild decreases in arterial pressure in C57BL/6J mice using the noninvasive tail-cuff method following 11 wk of TAC. Terminals measurements of ventricular pressure could have been performed through LV catheterization, but the additional stress of anesthesia and arterial catheterization likely would have exacerbated the impairment in mitochondrial oxidative metabolism.

The mechanism for the increased mortality in fructose diet-fed sham animals is unclear. No evidence of cardiac-related death was found based on EF (Fig. 3c) or the expression of atrial naturietic factor or MHC-β (Table 4). A possible explanation for the mortality seen in the fructose diet-fed sham group is renal damage. It has recently been shown in rats that 60% fructose consumption over 8 wk was associated with kidney hypertrophy, glomerular hypertension, cortical vasoconstriction, and a decreased glomerular filtration rate (35). In the present study, 61% fructose diet feeding was maintained for 16 wk, possibly inducing irreversible renal damage and subsequent death due to renal failure. Future studies involving chronic fructose diet feeding should assess structural and functional aspects of the kidney to provide evidence for this hypothesis.

An important finding in this study was the cardioprotective effects of a high-starch diet during chronic pressure overload. Cornstarch, the primary source of carbohydrate in the starch diet used in this study, contains mostly amylopectin. Having a branched-chain configuration, amylopectin is more susceptible to degradation by α-glucosidases compared with straight-chain amylase, suggesting a relatively high glycemic index for the starch diet and theoretically greater cardiac growth. However, TAC animals consuming the starch diet exhibited no increase in heart mass or contractile dysfunction compared with sham animals. The beneficial effects of high-starch diet feeding may be related to overall myocardial oxidative capacity, as the activities of MCAD and CS were maintained in starch diet-fed TAC mice compared with fructose diet-fed TAC mice (Fig. 4).

Further studies are needed to determine how dietary manipulation during pressure overload affects substrate preference and the hypertrophic response.

The blunting of cardiac hypertrophy and contractile dysfunction with high-starch diet feeding may also be species dependent. A concurrent feeding study in our laboratory involving the rat abdominal aortic constriction model of pressure overload found similar increases cardiac mass, LVESV, and LVEDV in animals fed either a high-sugar or high-starch diet (M. K. Duda, K. M. O’Shea, and W. C. Stanley, unpublished data). However, compared with a high-starch diet, feeding a high-sugar diet to hypertensive Dahl salt-sensitive rats decreased EF and increased mortality (38), consistent with the findings of the present investigation. Taken together, those

Table 5. Western blot densitometry of Akt, phospho-Akt (Ser473), AMPK, and phospho-AMPK (Thr472) in the heart

<table>
<thead>
<tr>
<th>Protein</th>
<th>Starch (n = 8)</th>
<th>TAC (n = 7)</th>
<th>STD (n = 8)</th>
<th>TAC (n = 7)</th>
<th>Fructose (n = 7)</th>
<th>TAC (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospho-Akt</td>
<td>0.89 ± 0.09</td>
<td>0.99 ± 0.11</td>
<td>1.00 ± 0.08</td>
<td>0.83 ± 0.06</td>
<td>0.88 ± 0.10</td>
<td>0.78 ± 0.03</td>
</tr>
<tr>
<td>Total Akt</td>
<td>0.97 ± 0.09</td>
<td>0.89 ± 0.06</td>
<td>1.00 ± 0.06</td>
<td>0.99 ± 0.06</td>
<td>1.09 ± 0.12</td>
<td>1.11 ± 0.12</td>
</tr>
<tr>
<td>Phospho-Akt/total Akt</td>
<td>0.92 ± 0.08</td>
<td>1.10 ± 0.06</td>
<td>1.00 ± 0.07</td>
<td>0.86 ± 0.08</td>
<td>0.87 ± 0.09</td>
<td>0.76 ± 0.09</td>
</tr>
<tr>
<td>Phospho-AMPK</td>
<td>0.86 ± 0.06</td>
<td>0.95 ± 0.04</td>
<td>1.00 ± 0.07</td>
<td>0.87 ± 0.06</td>
<td>0.88 ± 0.03</td>
<td>0.93 ± 0.02</td>
</tr>
<tr>
<td>Total AMPK</td>
<td>1.10 ± 0.18</td>
<td>1.04 ± 0.09</td>
<td>1.00 ± 0.06</td>
<td>0.99 ± 0.09</td>
<td>1.06 ± 0.07</td>
<td>1.17 ± 0.10</td>
</tr>
<tr>
<td>Phospho-AMPK/total AMPK</td>
<td>0.84 ± 0.11</td>
<td>0.92 ± 0.08</td>
<td>1.00 ± 0.08</td>
<td>0.89 ± 0.06</td>
<td>0.83 ± 0.06</td>
<td>0.81 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data are presented as fold changes in protein expression relative to the STD diet-fed sham group (in arbitrary units). AMPK, AMP-activated protein kinase.
studies imply strain- and species-dependent effects of diet on cardiac remodeling and survival during pressure overload.

The disparate effects of fructose diet compared with starch diet consumption on the response to pressure overload suggest that the two diets act through different mechanisms. In other words, the beneficial effects of the starch diet may be mediated by activation of cardioprotective pathways [as suggested by improved activity of mitochondrial enzymes (Fig. 4)] and by reduced stimulation of maladaptive cardiac responses activated by fructose diet feeding. High-sugar diets, in addition to being potentially damaging to renal function (35), may increase the production of proinflammatory cytokines (6, 7), which are important predictors of mortality in HF patients (2). While the present study establishes that a high-starch diet elicits a more favorable cardiac response to pressure overload than a high-sugar diet, additional discovery-based experimentation (e.g., gene chip and proteomic analyses) is needed to identify the mechanism(s) responsible for the dramatic differences between the two diets.

In summary, the findings of the present study demonstrate that during chronic pressure overload, a high-starch diet prevents cardiac growth and remodeling while a diet high in simple sugar increases mortality and molecular markers of HF. Although current dietary recommendations suggest a high-carbohydrate/low-fat diet (21) and suggest minimizing the intake of sugar, there is nominal experimental evidence indicating that the risk for LVH and HF is affected by sugar consumption, particularly in the setting of hypertension. Our findings suggest that increasing sugar consumption has profound effects on the remodeling and contractile performance of the heart in response to pressure overload. The mechanism for these effects, however, remains unknown. Future studies should involve the use of transgenic mice, such as the cardiac insulin receptor knockout mouse, to target whether alterations in insulin signaling impact the development of LVH and the progression to HF.

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GRANTS

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