A high-fructose diet worsens eccentric left ventricular hypertrophy in experimental volume overload

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Bouchard-Thomassin A-A, Lachance D, Drolet M-C, Couet J, Arsenault M. A high-fructose diet worsens eccentric left ventricular hypertrophy in experimental volume overload. Am J Physiol Heart Circ Physiol 300: H125–H134, 2011. First published October 22, 2010; doi:10.1152/ajpheart.00199.2010.—The development of left ventricular (LV) hypertrophy (LVH) can be affected by diet manipulation. Concentric LVH resulting from pressure overload can be worsened by feeding rats with a high-fructose diet. Eccentric LVH is a different type of hypertrophy and is associated with volume overload (VO) diseases. The impact of an abnormal diet on the development of eccentric LVH and on ventricular function in chronic VO is unknown. This study therefore examined the effects of a fructose-rich diet on LV eccentric hypertrophy, ventricular function, and myocardial metabolic enzymes in rats with chronic VO caused by severe aortic valve regurgitation (AR). Wistar rats were divided in four groups: sham-operated on control diet (SC; n = 13) or fructose-rich diet (SF; n = 13) and severe aortic regurgitation fed with the same diets [aortic regurgitation on control diet (ARC), n = 16, and aortic regurgitation on fructose-rich diet (ARF), n = 13]. Fructose-rich diet was started 1 wk before surgery, and the animals were euthanized 9 wk later. SF and ARF had high circulating triglycerides. ARC and ARF developed significant LV eccentric hypertrophy after 8 wk as expected. However, ARF developed more LVH than ARC. LV ejection fraction was slightly lower in the ARF compared with ARC. The increased LVH and decreased ejection fraction could not be explained by differences in hemodynamic load. SF, ARC, and ARF had lower phosphorylation levels of the AMP kinase compared with SC. A fructose-rich diet worsened LV eccentric hypertrophy and decreased LV function in a model of chronic VO caused by AR in rats. Normal animals fed the same diet did not develop these abnormalities. Hypertriglyceridemia may play a central role in this phenomenon as well as AMP kinase activity.

chronic left ventricular volume overload (VO) causes severe left ventricular dilatation and eccentric hypertrophy. This type of left ventricular hypertrophy is encountered mainly in patients with valvular diseases such as chronic mitral or aortic valve regurgitation (AR). AR is associated with a long asymptomatic period during which the left ventricle (LV) progressively dilates and hypertrophies. In parallel with the LV dilatation, systolic function slowly decreases and symptoms eventually appear (4, 5). Although it is not the most frequent valvular disease in Western countries, it has been estimated based on the Framingham study that 13% of the population suffer from AR of varying degrees of severity (40).

No drug treatment has proved to be effective to decrease morbidity or mortality or delay the evolution toward heart failure or valve replacement surgery in patients with chronic VO from valve disease (5). The search for an effective treatment is still ongoing. Patient lifestyle has a significant impact on the evolution of many cardiac diseases. Whereas good habits such as exercising and eating low fat/sugar diets seem beneficial, a lack of physical activity and eating imbalanced diets may act in the opposite way. The impact of diet and exercise on the evolution of VO cardiomyopathy has received little attention. We have recently shown that exercise could improve survival, LV diastolic function, and heart rate variability and reduce myocardial fibrosis in a rat model of severe AR (21, 22). A diet with a high glycemic load is strongly associated with an increased risk of coronary heart disease (9). It has been suggested that the current high prevalence of the metabolic syndrome in the population may be a consequence of the increasing use of high-fructose corn syrup and sucrose by the food industry (41). Previous studies have reported that a fructose-rich diet fed to rats will eventually lead to the development of metabolic abnormalities sharing many similarities with the human metabolic syndrome (12, 27). This type of diet has also been shown to increase cardiac dysfunction and mortality in an animal model of LV pressure overload with concentric left ventricular hypertrophy (8, 27, 37, 38). The potential impact of a high-fructose diet on the progression of VO cardiomyopathy has never been explored. Therefore, this study was designed to assess the impact of a high-fructose diet on the development of eccentric left ventricular hypertrophy and its impact on ventricular function in rats with severe chronic left ventricular VO from severe AR.

METHODS

Animals, Adult male Wistar rats were purchased from Charles River (Saint-Constant QC, Canada) and divided into four groups as follows: 1) sham-operated animals on control diet (SC; n = 13), 2) AR control diet (ARC; n = 16), 3) sham on high-fructose diet (SF, n = 12; 60% fructose diet; Catalog No. TD.89247; Harlan Teklad, Madison, WI), and 4) AR on high-fructose diet (ARF; n = 12). The animals were maintained on either the control diet (Purina Rat Chow No. 5075) containing 4.5% fat, 18.5% protein, and 57.3% carbohydrate (41.2 g/kg from starch; 4.0 kCal/g) or the 60% fructose diet...
containing 5.2% fat, 18.3% protein, and 60.4% carbohydrate (60 g/kg from fructose; 3.6 kCal/g). The high-fructose diet was started 1 wk before the surgery in both SF and ARF groups and continued for 8 wk until euthanized. Food consumption was evaluated at midprotocol by weighing consumed food pellets every day for a week and then averaged for a day. The protocol was approved by the Université Laval’s Animal Protection Committee and followed the recommendations of the Canadian Council for Laboratory Animal Care.

Aortic regurgitation. Severe AR was induced by retrograde puncture of the aortic valve leaflets as previously described (2, 30). A complete echocardiographic exam was performed 2 wk after AR induction and the day before euthanasia 8 wk later. At the end of the protocol, animals were euthanized, hearts were quickly dissected, and all cardiac chambers were weighed. LV was snap-frozen in liquid nitrogen and kept at −80°C for further analysis. All euthanasia were scheduled at similar times of the day in the fed state to avoid circadian variations in metabolism. Lungs, liver, and abdominal fat were rapidly collected and weighed. Blood samples were taken for the measurement of glucose, triglycerides, insulin, leptin, and adiponectin levels in nonfasting animals.

Table 1. Euthanasia data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SC</th>
<th>SF</th>
<th>ARC</th>
<th>ARF</th>
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<tr>
<td>n</td>
<td>13</td>
<td>12</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Body Weight, g</td>
<td>578 ± 11.8</td>
<td>550 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>577 ± 11.3</td>
<td>561 ± 10.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Tibial length, mm</td>
<td>60.6 ± 0.35</td>
<td>61.0 ± 0.35</td>
<td>61.4 ± 0.43</td>
<td>60.9 ± 0.37</td>
</tr>
<tr>
<td>Heart, mg</td>
<td>1.367 ± 40.8</td>
<td>1.312 ± 33.0</td>
<td>1.895 ± 37.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.181 ± 67.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LV, mg</td>
<td>929 ± 22.7</td>
<td>912 ± 25.9</td>
<td>1.398 ± 23.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.568 ± 58.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ilV, mg/g</td>
<td>1.6 ± 0.03</td>
<td>1.7 ± 0.05</td>
<td>2.4 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Right ventricle, mg</td>
<td>10.7 ± 0.26</td>
<td>11.7 ± 0.36</td>
<td>19.6 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.6 ± 0.92&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Left atrial, mg</td>
<td>37.1 ± 3.59</td>
<td>39.8 ± 3.61</td>
<td>67.9 ± 5.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.3 ± 4.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lungs, g</td>
<td>2.4 ± 0.25</td>
<td>2.4 ± 0.19</td>
<td>3.3 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Retroperitoneal fat, g</td>
<td>9.3 ± 0.90</td>
<td>10.3 ± 1.28</td>
<td>6.4 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.6 ± 0.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals per group. SC, sham-operated group on control diet; SF, sham-operated group on high-fructose diet; ARC, aortic valve regurgitation (AR) group on control diet; ARF, aortic valve regurgitation group on high-fructose diet; LV, left ventricle; ilV, indexed left ventricular weight. Two-way ANOVA analysis: *P < 0.05 vs. control diet groups and **P < 0.01 and ***P < 0.001 vs. sham-operated animals. If interaction between AR and diet was found to have a P value below 0.05, a Bonferroni post-test was conducted: *P < 0.05, **P < 0.01, and ***P < 0.001 vs. control diet corresponding group.

Fig. 1. Animal daily food intake (in kCal per day; A) and plasma levels of glucose (B), triglycerides (C), insulin (D), leptin (E), and adiponectin (F) at the time of euthanasia. Results are expressed as means ± SE (n = 15 per group). Two-way ANOVA analyses are displayed to the right of each panel. AR, aortic valve regurgitation.
If interaction between AR and diet was found to have a dilution of a representative cDNA sample to confirm the efficiency of representative RNA sample (no RT control), and a series of 10-fold one tube with water only (no template control), one tube with a (preoptimized specific primer pairs from Qiagen). Each run included QuantiTect SYBR Green PCR kit and QuantiTect Primer Assays thermal cycler (Corbett Life Science, Sidney, Australia), using the in duplicate (technical duplicates) by Q-PCR in a Rotor-Gene 6200/l of initial RNA). Diluted cDNA (1.25 ng) was amplified water before amplification (with the final concentration corresponding was converted to cDNA using the QuantiTect Reverse Transcription stored frozen in RNAlater (Ambion, Austin, TX) were homogenized 32, 33).

Hemodynamic measurements. Aortic pressures, LV end-diastolic pressures, and dP/dt (positive and negative change in pressure over time) were measured invasively using a dedicated 2F impedance pressures, and dP/dt (positive and negative change in pressure over time) were measured invasively using a dedicated 2F impedance catheter (Millar Instruments, Houston, TX) under 1.5% isoflurane anesthesia just before euthanasia as previously described (21, 22, 32, 33).

Analysis of mRNA accumulation by quantitative RT-PCR. Tissues stored frozen in RNAlater (Ambion, Austin, TX) were homogenized in TRIzol (Invitrogen, Burlington, Ontario, Canada) using a Polytron according to the standard TRIzol procedure. Fifty nanograms of RNA was converted to cDNA using the QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA), a procedure that includes a genomic DNA elimination step. The cDNA obtained was further diluted 10-fold with water before amplification (with the final concentration corresponding to 0.25 ng/μl of initial RNA). Diluted cDNA (1.25 ng) was amplified in duplicate (technical duplicates) by Q-PCR in a Rotor-Gene 6200 thermal cycler (Corbett Life Science, Sydney, Australia), using the QuantiTect SYBR Green PCR kit and QuantiTect Primer Assays (preoptimized specific primer pairs from Qiagen). Each run included one tube with water only (no template control), one tube with a representative RNA sample (no RT control), and a series of 10-fold dilutions of a representative cDNA sample to confirm the efficiency of the amplification reaction.

The quantification of gene expression was based on the −2ΔΔCt method (24). Briefly, mean Ct values of technical duplicates for each gene of interest were subtracted from the mean Ct value (hence ΔCt) of the control housekeeping gene cyclophilin 1. The differences in the mean ΔCts between groups of rats (ΔΔCt) allow the calculation of relative levels of induction/repression of genes of interest.

Enzyme activity determinations. LV samples were kept at −80°C until assayed for maximal (Vmax) enzyme activities. Small pieces of LV (20–30 mg) were homogenized in a glass-glass homogenizer with 39 volumes of ice-cold extracting medium, pH 7.4, containing (in mM) 250 sucrose, 10 Tris-HCl, and 1 EGTA. Hydroxyacyl-Coenzyme A dehydrogenase (HADH) and citrate synthase (CS) enzyme activities were estimated by the reduction of NADP to NADPH in a spectrophotometer with wavelength set to 340 nm for the citric acid cycle, complex I for the respiratory chain, and HADH for fatty acid β-oxidation (25). The method for measuring creatine kinase (CK) activity in cardiac tissues was adapted from a protocol provided by Sigma-Aldrich (details below), with the inclusion in the assay buffer of dithiothreitol for the reactivation of CK and NaF for the inhibition of adenylate kinase (28). Immediately before assay, homogenates were diluted 1/80 in cold extraction buffer, and 5 μl of those diluted samples were then put into 195 μl assay buffer containing 42 mM glycylglycine (pH 7.4), 0.017% bovine serum albumin, 14 mM phosphocreatine, 1.4 mM adenosine diphosphate, 34 mM glucose, 0.4 mM β-NAD, 4.5 mM magnesium acetate, 20 mM dithiothreitol, 25 mM NaF, 10 U/ml hexokinase, and 33 mM/ml glucose-6-phosphate dehydrogenase. Absorbance at 340 nm was read at 30°C with readings every 15 s for 15 min. The slope of the linear part of the absorbance curve was used to calculate enzyme activity, which was reported as millimoles optical density per minute per milligrams protein. The activity of the complex I (NADH-ubiquinone oxidoreductase) was

### Table 2. Echocardiography data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SC</th>
<th>SF</th>
<th>ARC</th>
<th>ARF</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-diastolic diameter, mm</td>
<td>8.5 ± 0.19</td>
<td>8.2 ± 0.22</td>
<td>10.6 ± 0.12†</td>
<td>10.9 ± 0.27†</td>
</tr>
<tr>
<td>End-systolic diameter, mm</td>
<td>3.9 ± 0.18</td>
<td>3.6 ± 0.16</td>
<td>5.6 ± 0.17†</td>
<td>6.3 ± 0.24†‡</td>
</tr>
<tr>
<td>Wall thickness</td>
<td>2.0 ± 0.10</td>
<td>1.8 ± 0.11</td>
<td>1.9 ± 0.08</td>
<td>2.0 ± 0.11</td>
</tr>
<tr>
<td>Posterior, mm</td>
<td>1.5 ± 0.05</td>
<td>1.6 ± 0.06</td>
<td>1.9 ± 0.04*</td>
<td>1.7 ± 0.09*</td>
</tr>
<tr>
<td>Relative</td>
<td>0.41 ± 0.018</td>
<td>0.42 ± 0.022</td>
<td>0.36 ± 0.011†</td>
<td>0.34 ± 0.012‡</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>79.4 ± 1.05</td>
<td>80.2 ± 1.53</td>
<td>72.0 ± 1.78†</td>
<td>66.5 ± 2.14‡</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>54.6 ± 1.09</td>
<td>56.0 ± 1.76</td>
<td>47.5 ± 1.70‡</td>
<td>42.4 ± 1.83‡</td>
</tr>
<tr>
<td>LV mass echo, mg</td>
<td>1,159 ± 43.9</td>
<td>1,011 ± 42.9</td>
<td>1,795 ± 45†</td>
<td>2,039 ± 74.8‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals per group. Relative wall thickness is (septal wall thickness + posterior wall thickness)/end-diastolic diameter. LV mass echo is estimated LV mass by the method of Devereux (Ref. 13). Two-way ANOVA analysis: *P < 0.05 and †P < 0.0001 vs. sham-operated animals.

### Table 3. Hemodynamic values

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>ARC</th>
<th>ARF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>411 ± 10.1</td>
<td>393 ± 6.0</td>
<td>389 ± 8.5*</td>
<td>380 ± 9.9*</td>
</tr>
<tr>
<td>Stroke volume, μl</td>
<td>286 ± 11.9</td>
<td>222 ± 12.9</td>
<td>445 ± 26.4†</td>
<td>461 ± 24.3‡</td>
</tr>
<tr>
<td>Cardiac output, ml min⁻¹</td>
<td>118 ± 5.7</td>
<td>88 ± 5.8</td>
<td>178 ± 12.4</td>
<td>170 ± 7.5‡</td>
</tr>
<tr>
<td>dp/dtmax</td>
<td>9.145 ± 549.2</td>
<td>7.772 ± 491.3</td>
<td>7.285 ± 74.0</td>
<td>7.579 ± 342.3</td>
</tr>
<tr>
<td>dp/dtmin</td>
<td>7.081 ± 543.3</td>
<td>8.238 ± 1058.8</td>
<td>5.734 ± 582.2</td>
<td>6.785 ± 676.1</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>11.6 ± 0.78</td>
<td>9.2 ± 1.51</td>
<td>17.3 ± 1.29*</td>
<td>15.4 ± 1.08*</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>130 ± 6.3</td>
<td>133 ± 7.4</td>
<td>119 ± 2.9*</td>
<td>128 ± 2.6*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>98 ± 4.3</td>
<td>99 ± 4.7</td>
<td>59 ± 3.2‡</td>
<td>63 ± 4.0†</td>
</tr>
</tbody>
</table>

Values are means ± SE of the indicated number (n) of animals per group with the exception of the dp/dt (dp/dtmax and dp/dtmin), maximal and minimal derivative of pressure over time, respectively) and LV end-diastolic pressure values, where n = 5. Measurements were obtained under inhaled 1.5% isoflurane anesthesia. Stroke volume is in LV outflow tract by pulsed Doppler. Cardiac output is stroke volume × heart rate. Two-way ANOVA analysis: *P < 0.05 and †P < 0.0001 vs. sham-operated animals.
were purchased from Cell Signaling Technology (Beverly, MA). Bands were visualized and quantified with a ChemiImager system (Alpha Innotech).

**Statistical analysis.** Results are presented as means ± SE unless specified otherwise. Intergroup comparisons were done using two-way ANOVA and using Bonferroni post-test when indicated. Statistical significance was set at a $P < 0.05$. Data and statistical analysis were performed using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego CA).

**RESULTS**

**Clinical data and animal characteristics.** All animals were alive at the end of the protocol. Fructose-fed rats (SF and ARF) had a slightly lower body weight compared with their respective controls (SC and ARC) at the end of the protocol, although
overall growth was similar as demonstrated by the comparable tibial lengths in all groups (Table 1). ARC had less retroperitoneal fat than the ARF animals. As illustrated in Fig. 1A, fructose-fed animals (SF and ARF) had a lower caloric intake than animals on control diet.

As expected, heart weight was strongly increased in both AR groups compared with the sham-operated groups. The ARF had an increased total heart weight compared with ARC. This was due to an increase in LV mass in the ARF group. Right ventricular, left atrial, and lung weights were also increased in ARC and ARF, but the diets did not affect these measurements.

Plasma glucose levels (Fig. 1B) were similar between all groups with the exception of the ARF group, which tended to have higher glucose levels ($P = 0.07$), but this difference did not reach statistical significance. Triglyceride levels were strongly increased in both fructose-fed groups (SF and ARF) as expected with this diet composition (Fig. 1C). Insulin, leptin, and adiponectin levels remained similar between groups (Fig. 1, D and E). There was a trend toward a diet-disease interaction for the insulin levels ($P = 0.06$), but again the difference did not reach statistical significance.

**Echocardiographic and hemodynamic data.** As expected, severe AR led to enlarged end-diastolic and end-systolic dimensions in both groups (ARC and ARF; Table 2). ARF had larger end-systolic diameters and slightly lower systolic ejection fraction than ARC. LV mass estimated by echo was significantly increased in ARF compared with ARC and therefore corroborated well with the direct measurement of heart weight at euthanasia.

AR severity was similar in both ARC and ARF groups (results not shown). Heart rate was slightly lower in the ARC and ARF groups. AR also resulted as expected in larger and similar forward stroke volumes and increased cardiac output in those groups (Table 3). End-diastolic LV pressure was significantly higher in ARC and ARF compared with their respective sham controls. There was, however, no clear diet effect on this parameter. There was no diet effect or diet-disease interaction for any of the measured hemodynamic parameters between the ARC and ARF groups.

**Markers of LV remodeling.** As illustrated in Fig. 2, the gene expression of two markers of LV hypertrophy (atrial and brain natriuretic factors (ANP and BNP)) increased in both AR groups. There was no diet effect or diet-disease interaction on these parameters.

Interstitial fibrosis is a late feature in our model (22, 32). Standard LV tissue staining for the quantification of fibrosis did not show any difference between groups (results not shown). The gene expression of pro-collagens type I and III and fibronectin were measured and are reported in Fig. 3. There was a clear disease effect toward an increase in the expression...
of pro-collagen I in the AR groups and a trend in the same direction for pro-collagen III ($P = 0.06$), but post hoc testing was not significant. We did not find any diet effect on pro-collagen I gene expression. There was, however, a clear diet effect, suggesting an increased expression of pro-collagen III in the fructose-fed animals, but again this did not reach statistical significance after post hoc testing of the ANOVA results. Fibronectin expression was unaffected in all four groups without any measurable effect of the diet or the disease.

**Myocardial metabolic enzymes.** Data analysis suggested a disease effect on the level of LV enzymatic activity of HADH, the complex 1 of the mitochondrial electron transport chain (ETC-1), and CK but not on CS or SDH/ETC-2 activities (Fig. 4). Post hoc analysis did not reveal any significant differences, however, for these activity levels. There were no significant diet effects on the enzymatic activities reported in Fig. 4. However, we did find a significant diet-disease interaction for the SDH/ETC-2 activity, and this increase in the ARF compared with the ARC was statistically significant. Total CK activity was lower in both AR groups (Fig. 4E), but no statistically significant diet effect or diet-disease interaction was found. Phosphofructokinase activity remained unchanged between all four groups (results not shown).

The increase in circulating triglycerides in fructose-fed animals (SF and ARF) was not accompanied by any changes in the mRNA levels of fatty acid transporters [FAT/CD 36 and carnitine palmitoyl transferases (Cpt)], although AR seemed to induce a slight decrease in Cpt2 gene expression, which did not reach statistical significance (Fig. 5, $A–C$). Glucose entry in the cardiac cell is mainly mediated by glucose transporters 1 and 4 (GLUT 1 and GLUT4). GLUT4 mRNA expression levels remained similar between SC and ARC animals. The fructose diet tended to increase this gene expression in SF and not in ARF group but this did not reach statistical significance (Fig. 5, $D$ and $E$). On the other hand, mRNA levels encoding for insulin-independent GLUT1 increased in both AR groups compared with SC rats. Again post hoc testing was not significant.

Peroxisome proliferator-activated receptor-α (PPAR-α) is a main regulator of fatty acid metabolism. In our AR animals, LV mRNA levels of PPAR-α were slightly reduced but the diet had no significant effect on this parameter (Fig. 5F).

The AKT/mTOR is a known prohypertrophic signaling pathway. We did not observe any modulation of this signaling pathway in the LVs of our animals as illustrated in Fig. 6. Although the total protein content of AKT and 4EBP1 (a downstream effector of mTOR) were different in the SF group compared with controls, this did not translate to the content of their phosphorylated form [AKT (Ser473) and 4EBP1 (Ser65)]. AR-induced LV hypertrophy was accompanied by a significant decrease in the content of the phosphorylated form of AKT and 4EBP1.

**Fig. 5.** Evaluation by real-time quantitative RT-PCR of the LV mRNA levels of several genes related to cardiac metabolism. Evaluation by real-time quantitative RT-PCR of the LV mRNA levels of 2 glucose transporters, GLUT 1 ($D$) and GLUT 4 ($E$); fatty acid transporters (FAT/CD36; $A$); carnitine palmitoyl transferase [Cpt1b ($B$) and Cpt2 ($C$)]; and peroxisome proliferator activator receptor-α (PPAR-α; $F$) are shown. Results are reported in arbitrary units as means ± SE ($n = 10–15$ per group). Two-way ANOVA analyses are displayed to the right of each panel.
of AMPK-α (Thr172). The fructose diet had a similar effect on this parameter. Activation of LKB1, an AMPK regulator, was not significantly affected by the diet or the disease (Fig. 7).

**DISCUSSION**

In this study, we show in that a relatively short exposition (8 wk) to a fructose-rich diet increases eccentric LVH and slightly decreases LV ejection fraction in rats with severe VO from AR. Rats can cope with this type of LV VO and tolerated severe LV dilation for a relatively long period with survival rates of more than 70% 6 mo post-AR induction (22, 32). The present protocol was relatively short-termed and evaluated the compensated phase of the disease when LV dilation is almost maximal (30), but systolic function remains in the normal range. Despite this relatively short exposition to the high-fructose diet, we report a clear increase in LVH in the AR rats. We previously reported a clear link between the extent of LV hypertrophy and survival in our model (22). In the present study the AR animals fed with the high-fructose diet for only 2 mo had a larger heart and a lower ejection fraction than the...
AR animals fed with the control diet. This suggests that in the longer term the fructose-fed AR animals would probably have a poorer survival. This issue needs to be addressed in a longer study.

Cardiac disease in patients is often accompanied by metabolic abnormalities such as dyslipidemia, obesity, hypertension, insulin resistance, or diabetes. The fructose-fed AR rat model provides an interesting glimpse at the impact of diet-induced metabolic abnormalities in the context LV hypertrophy, but this type of diet has never been studied in a model of chronic LV VO with eccentric LVH (27). The ARF animals were not only hypertriglyceridemic but also had more peritoneal fat and a tendency for higher blood glucose levels and higher systolic blood pressure than those from the ARC group. The sham animals fed a high-fructose diet (SF) had similar metabolic abnormalities and hypertriglyceridemia than the ARF but they did not develop any LVH, LV dilatation, or dysfunction and for an increase in pro-collagen III expression in the myocardium of the animals after only 8 wk. We observed decreased levels of AMPK activation in SF and both AR groups. This observation may be important.

The accumulation of collagen is a late feature in our AR model and only occurs after 6–9 mo (22, 31, 32). Therefore, we did not expect to find any significant changes in collagen content in the myocardium of the animals after only 8 wk. We observed a trend toward an increase in pro-collagen I expression and for an increase in pro-collagen III expression in the fructose groups compared with the ones fed with the standard diet. It is likely that this would translate into an increase in myocardial fibrosis after a longer follow-up and maybe an earlier deterioration of diastolic function in the ARF rats. This will have to be evaluated in longer protocols.

LVH and heart failure are usually associated with a shift from normal fatty acid to glucose as the preferred myocardial fuel (36). In our model, this shift was not clearly present after 8 wk. Total CK, the complex 1 of the electron transport chain as well as SDH/ECT-2 enzymatic activities were reduced in AR rats, suggesting a possible early alteration of mitochondrial function in these animals. Surprisingly, SDH activity seemed restored in AR animals on the fructose diet. The SDH/ECT-2 links the Krebs cycle to the electron transport chain (3). On one hand, this may be seen as a positive effect of the fructose diet by maintaining normal levels of SDH activity in the Krebs cycle. On the other hand, if the ETC function is impaired in the heart of AR animals, an increase in complex II activity by the fructose diet could be associated with an increase in reactive oxygen species production (19). This protocol, unfortunately, was not designed to test this hypothesis.
The impact of the fructose diet on some myocardial enzymatic activities seemed different in AR animals compared with sham controls. The sham-operated animals on the fructose diet did not develop any hypertrophy or sign of LV dysfunction. How the dilated and hypertrophied left ventricle adapts to the high-fructose diet compared with a normal left ventricle and why it develops more hypertrophy remain a mystery. AR is associated with a decreased gene expression of PPAR-α, which is known to stimulate fatty acid oxidation (34). The overabundance of circulating triglycerides combined with a lack of increase in fatty acid oxidation by the heart could possibly lead to myocardial lipotoxicity, but this remains a hypothesis to be confirmed. The fructose diet slightly increased FAT/CD36 expression in both sham and AR animals. We previously observed that myocardial lipoprotein lipase activity remained unchanged after 6 mo in AR animals (unpublished observation). We hypothesize that the myocardium placed in presence of an excess of fatty acid substrate with a similar or reduced capacity for β-oxidation may develop lipotoxicity (6).

**Study limitations.** The results of this study have to be viewed in light of some limitations. Rodent heart metabolism may differ in some aspects from human heart metabolism. Substrate utilization was not directly assessed in vivo. The high-fructose diet had a slightly higher fat content (5.2% vs. 4.5%) and lower caloric content (3.6 kcal/g vs. 4.0 kcal/g) compared with the control diet. The impacts of a longer exposition to the abnormal diet have to be evaluated in longer protocols. Other signaling pathways potentially involved need to be investigated in more detail.

**Conclusions**

The results of this study show that a short exposition (8 wk) to a high-fructose diet is sufficient to worsen LV eccentric hypertrophy and LV function in rats with VO due to severe AR. Exposing AR rats to this high-fructose diet resulted in hypertriglyceridemia, a higher retroperitoneal fat content, and a trend for higher glycemia and higher systolic blood pressure than those fed a normal diet. Put together, these results suggest a trend for higher glycemia and higher systolic blood pressure in rats already suffering from a chronic stress such as VOs. The exact mechanisms involved and consequences for the heart will need to be explored in longer studies. Our current findings in conjunction of those of other authors (37–39) working on LVH pressure-overload models strongly point toward a deleterious role of high fructose consumption in subjects with concentric and eccentric LVH.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


