Cardiac and metabolic changes in long-term high fructose-fat fed rats with severe obesity and extensive intramyocardial lipid accumulation

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Axelsen LN, Lademann JB, Petersen JS, Holstein-Rathlou NH, Ploug T, Prats C, Pedersen HD, Kjølbye AL. Cardiac and metabolic changes in long-term high fructose-fat fed rats with severe obesity and extensive intramyocardial lipid accumulation. Am J Physiol Regul Integr Comp Physiol 298: R1560–R1570, 2010. First published March 31, 2010; doi:10.1152/ajpregu.00392.2009.—Metabolic syndrome and obesity-related diseases are affecting more and more people in the Western world. The basis for an effective treatment of these patients is a better understanding of the underlying pathophysiology. Here, we characterize fructose- and fat-fed rats (FFFRs) as a new animal model of metabolic syndrome. Sprague-Dawley rats were fed a 60 kcal/100 kcal fat diet with 10% fructose in the drinking water. After 6, 12, 18, 24, 36, and 48 wk of feeding, blood pressure, glucose tolerance, plasma insulin, glucose, and lipid levels were measured. Cardiac function was examined by in vivo pressure volume measurements, and intramyocardial lipid accumulation was analyzed by confocal microscopy. Cardiac AMP-activated kinase (AMPK) and hepatic phosphoenolpyruvate carboxykinase (PEPCK) levels were measured by Western blotting. Finally, an ischemia-reperfusion study was performed after 56 wk of feeding. FFFRs developed severe obesity, decreased glucose tolerance, increased serum insulin and triglyceride levels, and an initial increased fasting glucose, which returned to control levels after 24 wk of feeding. The diet had no effect on blood pressure but decreased hepatic PEPCK levels. FFFRs showed significant intramyocardial lipid accumulation, and cardiac hypertrophy became pronounced between 24 and 36 wk of feeding. FFFRs showed no signs of cardiac dysfunction during unstressed conditions, but their hearts were much more vulnerable to ischemia-reperfusion and had a decreased level of phosphorlated AMPK at 6 wk of feeding. This study characterizes a new animal model of the metabolic syndrome that could be beneficial in future studies of metabolic syndrome and cardiac complications.

metabolic syndrome; glucose intolerance; diabetes; animal model; myocardial contractile performance

THE HUMAN METABOLIC SYNDROME is a complex metabolic disorder influenced by genetic and environmental factors (32). The dramatic increase in patients with metabolic syndrome recently seen in Western societies is most likely due to alterations in lifestyle, which includes increased consumption of high-calorie diets and decreased physical activity.

High-fat diets have been widely used to generate obese rodent models, and several studies have revealed that, in addition to obesity, high-fat diets also promote hyperglycemia, dyslipidemia, and whole body insulin resistance [for recent review, see Buettner et al. (3)]. Based on these experiences, it is generally accepted that high-fat diets can be used to generate valid rodent models of the metabolic syndrome with obesity, dyslipidemia, and insulin resistance. However, as reviewed by Buettner et al. (3), the fat content and fat composition of the diet, as well as the feeding period and strain of rat or mice used, varies markedly between different studies, and these factors significantly impact the outcome.

High-fructose and high-sucrose diets have also been used to induce metabolic and physiological alterations in rodents, whereas high glucose feeding does not seem to induce the same pronounced metabolic alterations (43). Both high-fructose- and high-sucrose-fed rats show increased fasting glucose and triglyceride levels, and high-fructose-fed rats develop glucose intolerance and insulin resistance (5, 6, 17, 18, 25). Furthermore, several studies have found that a diet rich in fructose or sucrose induces hypertension in rats (11, 26, 33, 40, 44, 45), whereas a few studies have reported that fructose does not affect blood pressure (BP) (1, 41). In contrast to the observed increased weight gain in high-fat-fed rats, high-fructose and high-sucrose diets do not induce increased weight gain (5, 6, 18). However, serum triglyceride levels, hyperinsulinemia, and glucose intolerance seem to be more pronounced in high-fructose-fed rats compared with high-fat-fed rats (18). Finally, high-cholesterol diets have also been found to increase total cholesterol, triglyceride, and low-density lipoprotein levels in rats and increase mean arterial pressure (14). However, as for high-fructose diets, high-cholesterol diets seem to have no effect on body weight in either mice or rats (10, 16). Based on these observations, it seems reasonable to conclude that high-fat-, high-carbohydrate-fed, and high-cholesterol-fed rodents develop different phenotypes of the metabolic syndrome. Even so, a high-fat diet (28, 29), a high-fructose diet (17, 27), a high-sucrose diet (5), and a high-cholesterol diet (10) have all been reported to induce cardiac contractile dysfunction in rats or mice.

Because high-fat-, high-cholesterol-, and high-carbohydrate (fructose and sucrose)-fed rodents present different characteristics of the metabolic syndrome, it is reasonable to expect that a combination of some of these diets potentially may induce a phenotype of the syndrome more similar to that observed in the majority of humans, i.e., a combination of obesity, hypertension, dyslipidemia, glucose intolerance, and insulin resistance. Furthermore, it can be hypothesized that the potential combination of obesity and hypertension, along with more severe metabolic changes, will accelerate the development of cardiac dysfunction. An animal model with such characteristics could be very beneficial to future research in the metabolic syndrome and its cardiac complications.

To date, only a few studies have examined the effects of a high-fat diet combined with high fructose (36, 38, 41), and the
feeding protocols vary between the different studies. Furthermore, a thorough long-term characterization of such a model has not been conducted. Therefore, we initiated this long-term study to characterize high-fructose and fat-fed rats (FFFRs) as a diet-induced rat model of metabolic syndrome and to investigate the potential metabolic and cardiac complications that this model might develop. To study the time-dependent development of different metabolic abnormalities and pathophysiological complications, rats were examined every 6 or 12 wk throughout the 48-wk feeding period.

**METHODS**

**Animal model.** All animal studies were performed according to the Guide for the Care and Use of Laboratory Animals, published by the United States National Institutes of Health (NIH publication No. 85–23, revised 1996), and approved by the Animal Experiments Inspectorate of the Danish Ministry of Justice. Four-week-old male Sprague–Dawley rats (Tacoon, LI, Skensved, Denmark) were stratified according to equal weight into two pools containing 10 groups each. One pool (n = 8–12/group) received normal chow (4% fat, no. 1324 DK; Altromin, Lage, Germany) and water, and the other pool (n = 8–20/group) received a high-fat diet (60 kcal/100 kcal saturated fat, no. D12492; Research Diets) with 10% fructose (no. F0127; Sigma-Aldrich, Brøndby, Denmark) in the drinking water. To avoid bacterial growth in the drinking water, citric acid was added to give a pH of 3.6 in both control and fructose water. All groups had free access to water and chow. Body weight was recorded weekly. Metabolic profile and BP were evaluated at 6, 12, 18, 24, 36, and 48 wk of feeding followed by pressure-volume assessment of cardiac function according to the procedures described below. Following 56 wk of feeding, an ischemia-reperfusion study was conducted in a subset of animals.

**Intravenous glucose tolerance test and BP measurements in conscious rats.** Five days before the intravenous glucose tolerance test (IVGTT), chronic catheters (sterile Tygon S-54-HL catheters with 0.76 mm outer diameter; Saint-Germain Performance Plastics, Akron, OH) were placed in the right femoral artery and jugular vein. On the following day, rats were conditioned for restrainers. On the day of the experiment, rats fasted overnight were placed in restrainers. BP was measured by connecting the arterial catheter to a pressure transducer (Edwards Lifesciences, Irvine, CA), and pressure signals were obtained by gently compressing the caudal caval vein. To avoid interference from the ECG electrodes on volume measurements obtained by the pressure-volume catheter, ECG electrodes were disconnected during pressure-volume measurements.

To correct for parallel conductance and derive absolute volumes from the conductance catheter, two intravenous injections of 60 μl 25% saline were given at the end of the experiment. All pressure-volume experiments were recorded and analyzed using IOX version 2.2 software (EMKA Technologies, Paris, France).

**Urine analyses and pathology.** After the pressure-volume studies, a cannula was inserted in the bladder for urine collection. Semiquantitative determination of protein and glucose content in the urine was performed using a Combur-Test strip (Roche, Hvidovre, Denmark). Finally, the heart, right kidney, lung, and liver were removed, trimmed for fat, and weighed.

**Biochemical analysis.** High-density lipoprotein (HDL)-cholesterol, total cholesterol, and triglyceride were measured using an automated spectrophotometer (model 912; Roche/Hitachi, Hvidovre, Denmark). HDL-cholesterol was measured in EDTA plasma using an HDL-cholesterol testing kit (no. 03030067 12; Roche), whereas total cholesterol and triglyceride were measured in serum using a cholesterol testing kit (no. 11489232; Roche) and a triglyceride testing kit (no. 1488899; Roche), respectively. Insulin levels were measured in plasma using an insulin ELISA kit (EZRMl-13K; Linco Research). All tests were performed according to the instructions of the kits.

**Intramyocardial lipid accumulation.** Left ventricular cardiac tissue was pinned down at resting length in petri dishes coated with Sylgard 184 (Dow Corning, La Hulpe, Belgium), incubated with Krebs Henseleit buffer containing 1 g/l proacine hydrochloride (no. P9879; Sigma), and subsequently fixed in 2% paraformaldehyde (no. 4005; Merck) with 0.15% picric acid (no. 709254; Bié & Berntsen) in 0.1 M Sorensen’s phosphate buffer (23 mM NaH2PO4, 7 mM Na2HPO4, pH 7.35) at room temperature for 30 min. The muscles were left for an additional 4.5 h in the fixative at 4°C. Bundles of cardiomyocytes were isolated under a stereomicroscope using fine forceps as previously described (31). Lipid droplets were stained with Bodipy 493/503 (no. D-3922; Invitrogen, Taastrup, Denmark) by incubating bundles of cardiomyocytes for 1 h in PBS containing 20 μg/ml Bodipy followed by washing in PBS. Cardiomyocytes were oriented longitudinally and mounted in Vectashield (no. H-1000; Vector Laboratories, Burlingame, CA) on a glass slide. Image stacks were collected with a TCS SP2 (Leica) confocal microscope through a HCX plan APO ×63 objective with a numerical aperture of 1.32. The z-distance between planes was set to 0.3 μm. Four consecutive optical planes were used to construct average projections, which were analyzed using MetaMorph software (version 6.1.0; Universal Imaging, San Francisco, CA). The amount of lipid per cardiomyocyte was obtained by quantifying Bodipy emission from a standardized region in single cardiomyocytes, as previously described for analysis of lipid droplets in soleus muscle fibers (31).

**Ischemia-reperfusion study.** The rats were anesthetized with a subcutaneous injection of 2 ml/kg of a 1:1:2 mixture of Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; Janssen-Cilag, Birkerød, Denmark)-Midazolam (5 mg/ml; Dumex-Alpharma, Copenhagen, Denmark)-distilled water. Tracheotomy was performed, and the rats were ventilated with room air using an Ugo Basile rodent ventilator (Comerio...
VA) (tidal volume = 6 ml; frequency = 60/min). The thorax was opened, and the aorta was exposed and cannulated as previously described (21).

After transfer to the perfusion apparatus (Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany), an incision was made in the left auricle. A fluid-filled balloon (size 7) (EMKA Technologies) was inserted through the left auricle in the LV to allow measurements of left ventricular pressure. The volume of the balloon was adjusted to give an end-diastolic pressure of 5 mmHg.

A ligature was placed around the left anterior descending coronary artery (LAD), and both ends of the ligature were passed through a small plastic tube enabling induction of ischemia by pressing the plastic tube against the heart and clamping the ends of the ligature. Two monophasic action potential (MAP) electrodes were placed in direct contact with the epicardial surface of the LV (one in the area at risk of infarction and one outside of the area at risk). In addition, two MAP electrodes were used to obtain unipolar ECG recordings by placing them a few millimeters from the surface of the LV.

When all electrodes were in place, the water bath was elevated to immerse the heart in 37°C Krebs-Henseleit solution. The hearts were perfused in the Langendorff mode at constant perfusion pressure (~60 mmHg) with a prewarmed (37°C) modified Krebs-Henseleit solution with the following composition (mmol/l): 118.0 NaCl, 4.7 KCl, 2 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, 2.0 sodium pyruvate, 24.9 NaHCO3, and 10.0 glucose, pH of 7.4–7.5, and an adequate oxygen content of the solution was obtained by continuously bubbling with carbogen (95% O2-5% CO2).

The experiment was initiated with 30 min of normal perfusion (the equilibration period). Regional ischemia was then introduced by tightening the ligature around the LAD for 30 min. Subsequently, the ligature was loosened, and the heart was reperfused for 60 min. At the end of the experiment, the ligature around the LAD was clamped again, and the heart was perfused with Evans Blue dye to evaluate the area at risk of infarction.

All Langendorff studies were recorded and evaluated using Notocord HEM version 3.5 software (Notocord Systems, Croissy sur Seine, France).

**Western blots.** Twenty to thirty milligrams of frozen heart (from 6, 24, and 48 wk of feeding) (n = 6–10/group) or liver tissue (from 18, 24, and 48 wk of feeding) (n = 6–11/group) were homogenized with a Polytron PT 3100 (Kinematica, Littau-Luzern, Switzerland) in 750 l of buffer [3% SDS, 10 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 5 mM EDTA, 2 mM Tris·HCl, pH 6.8, and “Complete protease inhibitor cocktail” tablets (Roche Applied Science, Hvidovre, Denmark)] at room temperature, briefly sonicated, and stored in aliquots at ~80°C. Protein concentrations were determined by the BCA assay (Sigma-Aldrich) using BSA as a standard.

For Western blot, 15 μg of protein were separated by SDS-PAGE on 4–15% Criterion gels (Bio-Rad, Copenhagen, Denmark) and electrophoretically transferred to polyvinylidene difluoride membranes in a tank buffer system (transfer buffer contained 48 mM Tris, 39 mM glycine, and 20% methanol). Membranes were blocked in 5% BSA plus 2.5% defatted milk powder in 10 mM Tris (pH 7.4) with 150 mM NaCl and incubated for 90 and 60 min with primary and
Table 1. Serum triglyceride, plasma HDL, and serum cholesterol levels in FFFR and control-fed rats after 48 wk of feeding

<table>
<thead>
<tr>
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<th>FFFR</th>
<th>Control</th>
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<tr>
<td>Triglyceride, mmol/l</td>
<td>1.72 ± 0.460</td>
<td>0.940 ± 0.067 ***</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.95 ± 0.192</td>
<td>2.035 ± 0.117 NS</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>4.50 ± 0.609</td>
<td>3.87 ± 0.251 NS</td>
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Values are presented as means ± SE. FFFR, fructose and fat fed rats; HDL, high-density lipoprotein. ***P < 0.001. NS, nonsignificant.

RESULTS

Body weight. Male Sprague-Dawley rats receiving a high-fat and fructose diet for 48 wk (FFFR) developed severe obesity (830.8 ± 17 g) relative to rats fed normal chow (621.8 ± 39 g) (Fig. 1). There was interaction (P < 0.0001) between duration of feeding and diet, and the difference between groups was statistically significant from 20 wk of feeding.

Glucose tolerance, insulin levels, and lipid profile. Fasting blood glucose levels were significantly increased in the FFFRs after 6, 12, and 18 wk of feeding (Fig. 2A). However, following 24, 36, and 48 wk of feeding, FFFRs had similar fasting blood glucose levels as control rats.

Urine glucose content was significantly increased in FFFRs after 12 and 18 wk of feeding (Fig. 2B), and the urine protein content increased with age in both FFFRs and control rats, but no difference was observed between the two groups (data not shown).

Glucose tolerance was dependent on both diet and duration of feeding (P < 0.0001), and it was significantly decreased in FFFRs compared with control-fed rats after 18, 36, and 48 wk of feeding (Fig. 2C). Furthermore, FFFRs showed increased fasting plasma insulin (P = 0.03 for effect of diet) (Fig. 2D) and triglyceride (P < 0.001) (Table 1) levels. No statistically significant difference in HDL-cholesterol and total cholesterol was found after 48 wk of feeding (Table 1).

BP and heart weight. Neither FFFRs nor control rats showed any change in mean arterial BP throughout the 48 wk of
feeding, and no statistically significant difference between the two groups was found at any time point (Fig. 3A).

Generally, heart weight, LV, and right ventricular weight were significantly increased in FFFRs compared with controls from 18 wk of feeding (Fig. 3, B-D), but interaction between diet and duration of feeding was only statistically significant for total heart weight and LV weight ($P < 0.0001$).

No difference in lung or kidney weight could be detected between the two groups (data not shown).

Liver weight and PEPCK protein levels. There was a statistically significant interaction ($P = 0.02$) between diet and feeding period on the weight of the liver (Fig. 4A), and there were macroscopic signs of liver steatosis in the FFFRs. The level of PEPCK was slightly lower in liver tissue from FFFRs compared with control. The decrease was statistically significant following 18 ($P < 0.001$) and 24 ($P < 0.01$) wk of feeding but not following 48 wk (Fig. 4B).

Intramyocardial lipid accumulation and in vivo left ventricular performance. Confocal fluorescence microscopy performed at 6, 12, 19, and 24 wk of feeding revealed a significant effect of diet on cardiac lipid accumulation ($P < 0.0001$). Cardiomyocytes from FFFRs showed highly increased lipid accumulation compared with control rats at all time points (Fig. 5). Heart rate was slightly higher in FFFRs compared with controls throughout the entire study ($P = 0.03$) (Fig. 6A). Both positive and negative $dP/dt_{max}$ for the LV were slightly increased in the FFFR group compared with controls throughout the 48 wk of feeding ($P = 0.005$ and $P < 0.001$, respectively) (Fig. 6, C and D), and the diastolic relaxation constant tau was slightly lower in the FFFR group compared with controls ($P = 0.02$) (Fig. 6E). However, ejection fraction and the end systolic pressure volume relation remained unchanged between the two groups (Fig. 6, B and F). In addition to the evaluated parameters of left ventricular performance presented in Fig. 6, stroke volume, cardiac output, maximal systolic pressure, and minimal diastolic pressure were also evaluated, but no differences were detected between the two groups at any time point (data not shown).

Cardiac AMPK levels. AMPK levels, i.e., both $\alpha_1$ and $\alpha_2$ isoforms, and AMPK phosphorylation [AMPK-P-(172Thr)] were measured in hearts from control and FFFRs following 6, 24, and 48 wk of feeding (Fig. 7). The AMPK level was unchanged between the control and FFFR groups at all evaluated time points (Fig. 7, A and B), whereas the level of phosphorylated AMPK was significantly decreased ($P < 0.01$) in hearts from FFFRs at 6 wk of feeding (Fig. 7C). However, following 24 and 48 wk of feeding, the level of phosphorylated AMPK was decreased to similar levels in both FFFRs and controls (Fig. 7C).

Cardiac sensitivity to ischemia and reperfusion. Figure 8 shows cardiac performance during ischemia and reperfusion, in isolated perfused hearts from FFFRs and control rats after 56 wk of feeding.

At baseline, isolated hearts from the FFFRs had statistically significant higher values of positive and negative $dP/dt_{max}$ ($P = 0.03$ and $P = 0.004$), higher developed left ventricular pressure ($P = 0.01$), and lower heart rate ($P = 0.045$) than control rats. The functional response to 30 min of regional ischemia was similar in the two groups, and so was the area at risk (data not shown). During reperfusion, all control hearts recovered to baseline levels, whereas seven of the nine FFFR hearts went into asystole during the 60-min reperfusion period ($P = 0.01$ for survival proportions between the two groups) (Fig. 8F).

DISCUSSION

A diet rich in fructose and fat induces metabolic syndrome in rats. The diet used in the present study caused pronounced obesity, impaired glucose tolerance, increased fasting serum triglyceride, increased insulin levels, and massive intramyocardial lipid accumulation in male Sprague-Dawley rats, which is in accordance with the World Health Organization definition of the metabolic syndrome (9). In addition, we found that the fasting glucose level was increased in FFFRs compared with control-fed rats at 6, 12, and 18 wk of feeding, and the urine glucose level was increased at 12 and 18 wk. The elevated fasting blood and urine glucose levels normalized over time and were similar to that in control rats after 24, 36, and 48 wk of feeding. The reason why blood glucose normalizes is unclear. We did not measure exact water intake during our study. However, judged from the amount of remaining fructose solution in the bottles when they were changed, it seemed as if the FFFRs consumed high amounts of fructose water during the first months of the feeding period and that their water and thus fructose intake decreased in the later part of the study period. A decreased intake of fructose could therefore contribute to the normalization of blood glucose. The change in glycemic state might also, at least partly, be explained by the gradual increase in
fasting insulin levels observed in the FFFRs throughout the study. Another possibility could be that, as adipose tissue increases in FFFRs throughout the study, so does the uptake of fructose by the adipose tissue, reducing the amount of fructose available for gluconeogenesis by the liver and consequently the blood glucose level. Also, the fructose and fat diet may lead to increased free fatty acid levels in the liver, which have been linked to hepatic insulin resistance and thereby an increased hepatic glucose production (42). This increased glucose production could be responsible for the high glucose levels seen within the first 18 wk of the study. Because more and more triglyceride accumulates in the liver and the animals develop nonalcoholic fatty liver disease (NAFLD), the liver’s ability to perform gluconeogenesis might be reduced, accounting for the fall in blood glucose. Even though NAFLD is believed to be a hepatic manifestation of the metabolic syndrome, and NAFLD patients present with many features of metabolic syndrome, they do not have elevated fasting glucose levels (30). In an attempt to clarify if the changes in blood glucose over time in FFFRs are connected to changes in gluconeogenesis by the liver, we measured hepatic levels of the rate-limiting enzyme for gluconeogenesis, PEPCK (2, 34). At 18 wk of feeding, where fasting glucose levels were increased in FFFRs, our study showed an ~20% decrease in hepatic PEPCK levels between control rats and FFFRs. This is in agreement with the recent finding that fasting hyperglycemia in type 2 diabetic patients is not associated with increased expression of PEPCK or glucose-6-phosphatase (35). At 24 wk of feeding, when fasting glucose levels had returned to normal in the FFFRs, hepatic PEPCK protein levels were still decreased in FFFRs compared with controls, whereas there was no statistically significant difference at 48 wk of feeding. Other studies have shown that 60 and 70% decreases in PEPCK protein levels result in only 33% and 20% reduction in gluconeogenic flux in canines (13) and transgenic mice (4), respectively. Therefore, we conclude that the changes in fasting glucose levels observed over time in our FFFRs cannot be explained by changes in hepatic PEPCK levels.

A high-fructose and fat diet does not induce hypertension. In this study where 10% fructose in the drinking water was given in combination with 60% fat in the diet for 48 wk, no effect on BP could be detected at any of the evaluated time points. Only a few other studies report that fructose does not affect BP in male Sprague-Dawley rats (1, 41), whereas most studies find that a diet rich in fructose induces hypertension (11, 26, 33, 40, 44, 45). A possible explanation for the discrepancies regarding the effect of a high-fructose diet on BP could be the different methods used for BP measurements. In most studies describing fructose-induced hypertension, the tail cuff method was used for BP measurements. In our study, we used chronically inserted catheters for BP measurements and found no effect on BP. In accordance with our observation, no effect of fructose feeding on BP was found in rats chronically instrumented with radiotelemetry BP transmitters (41). Therefore, we hypothesize...
that high-fructose-fed rats are more sensitive to the stress associated with tail cuff BP measurements relative to control-fed rats. This theory is further supported by the finding that high-fructose-induced hypertension observed using the tail cuff method can be completely abrogated by chemical sympathectomy (44). Our use of restrainers for the BP measurements may also have caused some degree of stress for the rats. However, to minimize the stress, our rats were conditioned for the restrainers on three consecutive days before experiments, and during BP recordings they were left alone and not handled by humans for 1 h. Even so, it cannot be excluded that other yet unknown factors might also influence the effect of a high-fructose diet on the development of hypertension.

FFFRs have unchanged cardiac function during nonischemic conditions but increased sensitivity to ischemia-reperfusion injury. In patients with metabolic syndrome, the heart typically has an increased mass, altered diastolic function, and the patients are prone to heart failure (15). In this study, the high-fructose and -fat diet also resulted in statistically significantly enlarged hearts. The enlargement of the LV seemed to be especially pronounced between 24 and 36 wk, indicating left ventricular hypertrophy. This is in accordance with previous findings showing that both high fat (28) and high fructose (26) are able to induce an increase in heart weight in rats. A necessary mediator of cardiac myocyte enlargement and thereby cardiac hypertrophy is protein synthesis. Activation of AMPK is known to inhibit protein synthesis associated with cardiac hypertrophy (7, 8), and high-fat feeding of rats has been found to impair both the expression and activity of AMPK in skeletal muscles (22). However, our data show that the expression of AMPK in cardiac tissue is unchanged in FFFRs compared with control-fed rats following 6, 24, and 48 wk of feeding. Furthermore, even though the amount of phosphorylated and thereby activated AMPK is significantly lower in the FFFRs compared with controls following 6 wk of feeding, there are no significant differences in phosphorylated AMPK following 24 and 48 wk of feeding. Therefore, the cardiac hypertrophy observed in the FFFRs cannot be explained by changes in total or activated AMPK.
FFFRs showed pronounced intramyocardial lipid accumulation already 6 wk into the study, and the lipid accumulation remained elevated throughout the study. Increased cardiac lipid accumulation has previously been associated with contractile dysfunction in obese Zucker rats (46, 47), and it has been found to be relatively common in obese and diabetic nonischemic heart failure patients (39). Despite the morphological alterations found in the hearts of the FFFRs, no decrease in cardiac function was observed at any of the evaluated time points. If anything, our data indicate a slightly improved systolic and diastolic function in the FFFRs, since both positive and negative dP/dt max were increased compared with the control group. This result was surprising, since a high-fat diet for only 8 wk (29) and a high-cholesterol-fructose diet for 15 wk (12) have been reported to induce a decreased cardiac contractile function in rats.

Because the FFFRs did not show a compromised cardiac function during unstressed conditions in vivo, an ischemia-reperfusion study was performed in isolated hearts from a subset of old animals after 56 wk of feeding. Four weeks of fructose feeding have previously been reported to protect against ischemia-reperfusion injuries in isolated perfused rat hearts (20), whereas the effect of fructose feeding on the outcome of left coronary artery ligation in vivo has been ambiguous (19, 27). In our isolated perfused hearts, the baseline values for positive and negative dP/dt max were higher in the FFFR group compared with controls, exactly as seen by the in vivo pressure-volume measurements. Heart rates were higher in the FFFR group compared with controls in vivo, but lower in the isolated hearts, suggestive of an increased sympathetic tone in FFFRs. During ischemia and reperfusion, the heart rate increased in the FFFR hearts but remained unchanged in control hearts. Beside the changes in heart rate, cardiac function decreased to the same extent in FFFR and control hearts during ischemia. However, during reperfusion, cardiac function improved in control hearts, whereas seven of nine FFFR hearts went into asystole. Therefore, in contrast to the uncompromised cardiac function found during unstressed conditions in vivo, our ischemia-reperfusion experiment clearly showed that long-term intake of high fructose in combination with high fat aggravates the outcome of an ischemic event. The incidence and severity of complications associated with ischemic events are known to be greater in the diabetic population, and they seem to be correlated to metabolic changes in the heart (23). However, our knowledge of the effects of obesity and diabetes on cardiac metabolism and function is still limited (23, 24), and future studies are needed to clarify the mechanisms behind the cardiac changes.

Strength and weaknesses of the study. This study offers a systematic long-term in vivo characterization of cardiac function in a diet-induced rat model of metabolic syndrome. To our knowledge, we are the first to look at the time-dependent changes in cardiac function and metabolic markers of metabolic syndrome using a combination of a high-fructose and high-fat diet. We have shown that the FFFR model develops several characteristics of metabolic syndrome along with hepatic changes and morphological alterations in the heart. The morphological cardiac changes were not associated with decreased cardiac function during nonischemic conditions, but the isolated hearts from the FFFRs were more vulnerable to ischemia-reperfusion injury compared with control rats. Therefore, it would have been an advantage to our study if we had performed the ischemia-reperfusion studies at all time points, enabling us to determine the timeline for the development of functional cardiac changes. Finally, it is important to acknowledge that the fat diet used for our FFFRs contained 60 kcal/100 kcal fat, which presumably is higher than what is generally consumed by humans with diet-induced metabolic syndrome. However, we chose this high-fat content hoping that it might accelerate any potential metabolic changes related to the intake of a high-fat diet.

For future studies using the FFFRs, an echocardiographic evaluation of heart function could be a significant advantage. Furthermore, given the observed changes in blood and urine glucose, it would also be valuable to measure the actual fructose-water intake. This information could give a better idea

![Fig. 7. A: AMP-activated kinase (AMPK) α1 protein levels. B: AMPK α2 protein levels. C: AMPK phosphorylated at 172Thr (AMPK-P172)-to-total AMPK protein levels in cardiac tissue from FFFR and control rats at 6, 24, and 48 wk of feeding. Values are presented as means ± SE.](http://ajpregu.physiology.org/)

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about the mechanism responsible for the drop in glucose levels seen between 18 and 24 wk of feeding.

Perspectives and Significance

The FFFR is a diet-induced animal model showing many of the characteristic features of metabolic syndrome seen in humans. The fat-fed or fructose-fed rat models, which are commonly used as diet-induced models of metabolic syndrome, present with different characteristics; increased serum triglyceride levels are only found in high-fructose-fed rats, whereas only a high-fat diet induces obesity (18). However, fructose-induced leptin resistance has been shown to accelerate high-fat-induced obesity (37). Furthermore, hyperinsulinemia and glucose intolerance seem to be more pronounced in high-fructose-fed rats compared with high-fat-fed rats (18). In contrast, the FFFRs present with a combination of obesity, decreased glucose tolerance, hyperinsulinemia, high plasma triglyceride levels, and initially increased fasting glucose. This, in combination with the highly increased intramyocardial lipid accumulation and an increased sensitivity to ischemia-reperfusion injury, makes the FFFRs an interesting model for future studies on diet-induced metabolic syndrome and cardiac complications. Furthermore, it might be possible to make the model even more similar to the human phenotype of metabolic syndrome by inducing hypertension. This could potentially be achieved by applying the feeding protocol to spontaneously hypertensive rats or by adding high salt to the feeding protocol. Finally, the model might also prove useful for studies of NAFLD or other diseases related to metabolic syndrome.

ACKNOWLEDGMENTS

We acknowledge Karin Larsen and Christina Hansen for excellent technical assistance as well as all animal technicians involved in the study. We also thank Jørgen F. Wojtaszewski for helpful discussion concerning the anti-AMPK antibodies.

Fig. 8. Hemodynamic parameters in isolated hearts from FFFR (n = 9) or rats fed a control diet (n = 3) for 56 wk, following a 30-min equilibration period (Eq), 5 and 29 min of ischemia, and 5, 30, and 60 min of reperfusion (rep) in Langendorff mode. A: heart rate. B: coronary flow. C: dP/dt max. D: −dP/dt max. E: developed left ventricular pressure (DLVP). F: percentage of hearts without asystole. Values are presented as means ± SE. The change in the FFFR group from 5 to 60 min of reperfusion is marked with a broken line since 7 of 9 hearts went into asystole during this period and only beating hearts are included in the data.
DISCLOSURES
At the time of the study, L. Axelsen, H. Pedersen, J. Petersen, and A. L.
Kjølbye were all full-time employees at Zealand Pharma A/S. J. B Lademann,
N.-H. Holstein-Rathlou, T. Ploug, and C. Prats have no conflict of interest to
disclose.

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