Cardiac function and lipid distribution in rats fed a high-fat diet: in vivo magnetic resonance imaging and spectroscopy

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1Laboratory of Molecular Imaging, Singapore Bioimaging Consortium, A*STAR, Singapore; 2Singapore Institute for Clinical Sciences, A*STAR, Singapore; 3Centre for Life Sciences, National University of Singapore, Singapore; 4Cardiovascular Research Institute, National University Hospital, Singapore; and 5Clinical Imaging Research Centre, NUS-A*STAR, Singapore

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Nagarajan V, Gopalan V, Kaneko M, Angeli V, Gluckman P, Richards AM, Kuchel PW, Sendhil Velan S. Cardiac function and lipid distribution in rats fed a high-fat diet: in vivo magnetic resonance imaging and spectroscopy. Am J Physiol Heart Circ Physiol 304: H1495–H1504, 2013. First published March 29, 2013; doi:10.1152/ajpheart.00478.2012.—Obesity is a major risk factor in the development of cardiovascular disease, type 2 diabetes, and its pathophysiological precondition insulin resistance. Very little is known about the metabolic changes that occur in the myocardium and consequent changes in cardiac function that are associated with high-fat accumulation. Therefore, cardiac function and metabolism were evaluated in control rats and those fed a high-fat diet, using magnetic resonance imaging, magnetic resonance spectroscopy, mRNA analysis, histology, and plasma biochemistry. Analysis of blood plasma from rats fed the high-fat diet showed that they were insulin resistant (P < 0.001). Our high-fat diet model had higher heart weight (P = 0.005) and also increasing trend in septal wall thickness (P = 0.07) compared with control diet rats. Our results from biochemistry, magnetic resonance imaging, and mRNA analysis confirmed that rats on the high-fat diet had moderate diabetes along with mild cardiac hypertrophy. The magnetic resonance spectroscopy results showed the extramyocellular lipid signal only in the spectra from high-fat diet rats, which was absent in the control diet rats. The intramyocellular lipids in high-fat diet rats was higher (8.7%) compared with rats on the control diet (6.1%). This was derived from the diet are stored as TGs in lipid droplets and those derived from the diet are stored as TGs in lipid droplets in both adipose and non-adipose tissues. TG accumulation is a major positive correlate of hypertension, insulin resistance, diabetes, pancreatic β-cell apoptosis, hypertrophic coronary artery disease, and cardiomyopathy (28, 29, 30). It is well known that excess TGs in non-adipose tissue leads to cellular dysfunction and cell death, a phenomenon known as lipotoxicity (14, 48). The accumulation changes the rate of lipid influx and its oxidation (14, 47, 48). In obese subjects adipose tissue releases increased amounts of nonesterified fatty acids, whereas the nonesterified fatty acid mobilization is suppressed in normal controls (8). Increased plasma insulin concentrations are correlated with increased left ventricular mass (39). Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) have been used in studies of dietary effects on cardiac function (2, 27). Recent advances in magnetic resonance (MR) technology for high magnetic field systems, including the use of phased array radiofrequency coils, motion compensation techniques, and optimal radiofrequency pulse sequences, now permit investigation of cardiac function and metabolism in greater detail (32).

Recent studies have shown altered lipid metabolism in patients with diabetes presaging diabetic cardiomyopathy (3). Skeletal muscle is another well-studied organ in diabetes and obesity, as it is the primary organ for glucose disposal in the body (10, 19). Specifically, the detection of two lipid pools in skeletal muscle with 1H NMR spectroscopic chemical shift difference between peaks of 0.2 parts per million (ppm) is attributed to the intra- and extramyocellular lipid (IMCL and EMCL, respectively) pools. Several groups have now established the fact that the IMCL is strongly associated with insulin resistance and diabetes in both rodents and humans (5, 15, 31, 42, 44). Recently, the presence of two lipid pools, including IMCL and EMCL, was demonstrated in cardiac muscle of patients with muscular dystrophy (34). On the other hand, very little is known about the metabolic changes in the myocardium that are associated with high-fat accumulation that putatively leads to cardiac dysfunction.

In this study we investigated myocardial fat accumulation in rats that were made obese on a high-fat diet. We used MRS, blood (plasma) biochemistry, mRNA analysis, and histology to quantify fat levels and distributions in the heart and correlated these findings with cardiac anatomy and function, as assessed by MRI.

METHODS

Animals. Fisher male rats were randomly divided into two groups at the age of 4 wk and housed in individual cages at 23°C. From 4 to 21 wk of age, the control group (n = 15) was fed with a 10% kilocalories including 17% protein, 73% carbohydrate, and 10% fat. The high-fat diet group (n = 13) was fed with 40% kilocalories
including 17% protein, 43% carbohydrate, and 40% fat to induce obesity. Animals were housed on a 12-h:12-h light-dark cycle, and they were given free access to the relevant diet and water. All experimental protocols were approved by the local biological resource center. At the age of 19 to 20 wk, one cohort of control (n = 5) and high-fat diet rats (n = 5) were subjected to MRS and MRI (referred to as “metabolic imaging”).

Myocardial imaging and spectroscopy. MRI and MRS measurements were performed on a 7-T Bruker ClinScan using a 72-mm volume transmit coil and a four-channel receive-array cardiac coil. Cine imaging was performed with a field of view of 50 × 50 mm and a matrix size of 256 × 256 pixels. Cardiac- and respiratory-gated long- and short-axis images of the heart were acquired for functional evaluation (27), and MRS localization was performed using sagittal, coronal, and transverse images. Left ventricular function was estimated by tracing contours of tissue and blood contrast by using a custom-developed Matlab program (1, 2). Localized point-resolved spectroscopy (PRESS) experiments were performed on the septum of the myocardium with a voxel size of 3 × 3 × 3 mm³ and time of repetition of 4.0 s, echo time of 13 ms, spectral width of 3,500 Hz with water suppression and outer-volume saturation of the water signal. In addition, unsuppressed water signal was acquired from the same location with identical experimental parameters. The 1H MR resonances from the n-methylene [(CH2)], of IMCL and EMCL were analyzed by theJava-based MR-user interface jMRUI software (36), using the AMARES algorithm, (41), and any residual water signal was removed by using the Henkel-Lanczos single-variable decomposition method.

Biochemical measurements. Body weight and food intake were measured weekly for 20 wk. Blood plasma glucose, TGs, and total cholesterol were measured at 20 wk (Quest Laboratories, Singapore). The oral glucose tolerance test (OGTT) was performed after overnight fasting. Glucose (2 g/kg) was orally administered to rats, and blood samples were collected from the tail vein at 0, 10, 30, 60, and 120 min. Plasma insulin was measured by commercial ELISA kit (Crystal Chem). The total areas under the glucose or insulin curves were determined from time 0 to 120 min after glucose administration. The numerical value of the product, fasting insulin concentration × fasting glucose concentration, was used as an index of insulin resistance.

Cardiac dissection. One week after OGTT, hearts were rapidly excised from the rats under anesthesia (2.5% vol/vol isoflurane), weighed, and sampled for both mRNA expression and TG measurements. Part of the tissue was immersed in RNAlater (Life Technologies) for mRNA expression analysis, and other parts were frozen in liquid nitrogen for cardiac TG measurements. Heart weight was normalized by using the length of the tibia (49).

mRNA expression analysis by real-time PCR. Total RNA was extracted using RNeasy Fibrous Tissue Mini Kit (QIAGEN, Hilden, Germany), and converted into cDNA with a High-Capacity cDNA Reverse Transcription Kit (Life Technologies). Gene expression was analyzed by ViiA 7 Real-Time PCR System (Life Technologies) using TaqMan Universal Master Mix I (Life Technologies) and primer-probe sets of TaqMan Gene Expression Assays (Life Technologies): medium chain acyl-CoA dehydrogenase (MCAD; Rn00566390_m1), carnitine palmitoyltransferase (CPTIb; Rn00682395_m1), cluster of differentiation 36 (CD36; Rn02115479_g1), uncoupling protein 2 (UCP2; Rn01754856_m1), binding immunoglobulin protein (BIP; Rn01457699_g1), and CCAAT/enhancer binding protein homologous protein (CHOP; Rn04929089_g1). Glyceroldehyde-3-phosphate dehydrogenase (GAPDH; Rn01775763_g1) was used as an endogenous control gene. Relative mRNA expression was calculated by ΔΔCT method.

Cardiac TG measurements. Rat hearts were homogenized in 0.6 ml of 6.7% wt/vol sodium sulfate solution. The lipids were extracted with 1 ml of hexane/isopropanol (3/2, vol/vol). The organic phase was collected and the lower phase was reextracted with 0.6 ml of hexane. The organic extracts were combined and dried under nitrogen and dissolved in Nonident-40/1,4-dioxane (1/1, vol/vol). TG concentration was determined by using a commercially available kit (TG-E test, Wako Pure Chemical Industries, Japan).

Electron microscopy and Oil Red O staining. After the final experiment the heart from a euthanized rat was fixed in 4% vol/vol glutaraldehyde and 10% vol/vol formalin in 0.1 M phosphate buffer overnight at pH 7.4. After 12 h of fixation, the ventricular septum was removed and prepared for electron microscopy and Oil Red O staining. For electron microscopy the tissue was fixed with 2% wt/vol osmium tetroxide and dehydrated through an ascending concentration series of ethanol and then embedded in Spur’s epoxy resin. The tissue samples were trimmed (~70 nm thickness) and contrasted with lead citrate (20). A Philips EM 208S transmission electron microscope (TEM) was used. Part of the tissue was fixed in 4% vol/vol glutaraldehyde and 10% formalin in 0.1 M phosphate buffer overnight at pH 7.4, postfixed in 1% wt/vol osmium tetroxide for 1 h, dehydrated in ethanol, and critical-point dried for scanning electron microscope (SEM) examination. Dried samples were coated with gold and examined using a JEOI JSM-5600LV microscope. The Oil Red O staining was performed as described in previous work (17, 23) with heart tissues of ~8 μm thickness and stained with filtered 0.3 g Oil Red O in isopropanol solution (60% vol/vol isopropanol and 40% water) for 30 min. Images were acquired using a Nikon Eclipse 90i light microscope fitted with an ultrahigh-resolution camera (DS-R1ii), and lipid droplets were imaged and quantified by NIS-elements Nikon software.

Statistical analysis. Statistical analysis was performed using the Mann-Whitney U-test for all biochemical and metabolic parameters. Median and interquartile ranges are reported, and P < 0.05 was considered significant. The body weight analysis was performed using repeated-measures ANOVA. All statistics were performed using IBM SPSS Statistics 20 software.

RESULTS

Table 1 shows the metabolic parameters measured, including body weight. The body weight growth curves for the animals are shown in Fig. 1. Initially at 4 wk of age, both groups had similar average body weight. Increases in the body weight were similar until 9 wk of age for both high-fat and control diet groups. From the 12th week onward, the growth in body weight was higher in the high-fat group. At 20 wk of age, the rats eating the high-fat diet showed a 39% increase in body weight relative to the controls (P < 0.001). Statistical analysis using repeated-measures ANOVA indicated that within groups, the weight gain over time was significant (P < 0.001), and between groups from the 11th week to the 20th week at each time-point weight gain in the high-fat group was significantly greater than the control diet group (P < 0.001).

Heart weight/tibia lengths (in mg/mm) in high-fat diet rats were higher (P = 0.005) compared with control diet rats. The overnight fasted plasma glucose (P = 0.03) and insulin (P = 0.03) concentrations were significantly higher in the high-fat diet group. The glucose concentration × insulin concentration (index of insulin resistance) was significantly higher in high-fat diet rats compared with the rats on the control diet (P < 0.001). The total cholesterol concentration TGs and FFAs were significantly higher in high-fat diet rats compared with the rats on the control diet (P < 0.001). Figure 2 shows the results from the OGTT measurements. Figure 2, A and B, shows the time course of plasma glucose and plasma insulin concentrations for control diet and high-fat diet rats. Figure 2, C and D, shows significant increases of both glucose and
insulin concentrations in high-fat diet rats compared with control diet rats as determined by the area under the curve from 0–120 min. The cardiac TGs in high-fat diet rats were significantly higher (1.2-fold) compared with the control diet rats, which was absent in the control diet. All spectra from this group of animals displayed only one set of lipid resonances from IMCL at 1.35 ppm. Figure 4B shows the localized MR spectra from the myocardium of rats on the high-fat diet. The myocardial spectra from all the high-fat diet rats showed two sets of lipid resonances including \(-\text{CH}_3\) at 0.85 ppm and \(-\text{CH}_2n\) at 1.35 ppm. Figure 4 shows a series of cine MRI images (sagittal, coronal, and transverse) for localization of the MRS voxel. Figure 4A shows the MR spectra obtained from the myocardium of rats fed the control diet. All spectra from this group of animals displayed only one set of lipid resonances including \(-\text{CH}_3\) at 0.85 ppm and \(-\text{CH}_2n\) at 1.35 ppm. We also observed the total creatine signal from the \(-\text{CH}_3\) groups (phosphocreatine and creatine) as a triplet centered at 3.03 ppm with one satellite beneath the trimethylamine resonance at 3.24 ppm. This occurred in both control and high-fat diet rats. The taurine signal was detected at 3.40 ppm in both groups. Figure 4, C and D, shows representative spectra of the unsuppressed water signal from both control and high-fat diet rats. Estimation of fat content (in %) relative to the unsuppressed water signal is shown in Fig. 5. The EMCL signal was observed only in the spectra from high-fat diet rats, which was absent in the control diet rats. The IMCL in high-fat diet rats was higher (8.7%) compared with rats on the control diet (6.1%).

**Electron microscopy, Oil Red O staining, and mRNA analysis.** Transmission electron microscopy (TEM) with a resolution of 2 \(\mu\)m was used to image IMCL and EMCL (fat) in the myocardium (Fig. 6). Figure 6A shows a cross section along the fibers in placebo rats in which there were few lipid droplets. Figure 6B shows a similar image from a high-fat diet rat where different sizes of lipid droplets are evident. Figure 6C shows a cross section perpendicular to the muscle-fiber direction in control rats where the lipid droplets were absent. Figure 6D shows the EMCL in a section perpendicular to the muscle-fiber direction (18, 20). To understand the orientation of fibers with reference to the magnetic field direction, we performed SEM. Figure 7A shows the sagittal MRI (z-y-plane) image indicating the voxel position where MRS acquisition was performed, and Fig. 7B shows the corresponding SEM image.
with the same orientation of the heart as in the MRI scanner. Figure 7C shows the expanded SEM image where the cardiac fiber orientation was predominantly along the main magnetic field z-direction. Similar observations have been made in earlier work on skeletal muscle (5, 15) (see DISCUSSION). Figure 8, A and B, shows the results of Oil Red O staining from both control and high-fat diet rats using a light microscope with ×40 objective lens. Both electron microscopy and Oil Red O staining confirmed the presence of large volumes of lipids inside the myocardium of high-fat diet rats. The quantification

Fig. 2. Oral glucose tolerance test for plasma glucose (A), plasma insulin (B), area under the curve (AUC) for 0–120 min for plasma glucose (C), and AUC for 0–120 min for plasma insulin (D). *P < 0.05, significant difference from control diet-fed group.

Fig. 3. Cine MRI images of sagittal (A), coronal (B), and transverse (C) sections from rat heart. The voxel position for localized magnetic resonance spectroscopy (MRS) is indicated by the square.
of histology images (Fig. 8C) showed an increase of lipids in high-fat diet rats ($P = 0.008$) compared with the control group.

Figure 9 shows the mRNA expression analysis by real-time PCR for both control and high-fat diet rats. There was increased expression of CD36 (1.8-fold), MCAD (1.4-fold), CPT1b (1.1-fold), and UCP2 (1.3-fold) in high-fat diet rats compared with control rat hearts. The BiP and CHOP expressions did not show any significant change between control and high-fat diet rat hearts.

**DISCUSSION**

Obesity can lead to the development of insulin resistance, cardiac hypertrophy consisting of myocardial function and metabolism (13). In this study, we investigated myocardial lipid accumulation in a moderate diabetic model of high-fat diet-induced obesity by MRI, MRS, electron microscopy, mRNA expression, and Oil Red O staining, combined with light microscopy in rats fed with a high-fat or a control diet. There was a significant increase in body weight in the high-fat diet animals at 20 wk. The fasting blood glucose and insulin concentrations indicated that the high-fat diet rats were insulin resistant (Table 1). There was also at least a threefold increase in plasma TGs in the high-fat diet rats. The OGTT measurements showed significant increases of both glucose and insulin...
in high-fat diet rats, confirming insulin resistance in our model. Furthermore, our data showed a significant increase in heart weight of high-fat diet rats that is evidence of cardiac hypertrophy. Earlier studies have shown that an increase in fatty acid uptake and its subsequent excessive β-oxidation are associated with diabetic cardiomyopathy including hypertrophy and cardiac dysfunction (11, 16, 25, 46).

In addition, excessive fatty acid oxidation is associated with production of reactive oxygen species, which in turn can lead to mitochondrial dysfunction resulting in cardiac hypertrophy (11, 16). Our mRNA analysis showed 1.8-fold increase in CD36 expression, indicating enhanced FFA transport in high-fat diet fed rats. We also observed significant increases in mRNA levels of CPT1b and MCAD, suggesting higher fatty acid oxidation rates. It is known that uncoupling lowers the production of mitochondrial ATP, contributing to the development of contractile dysfunction in type 2 diabetes (40). In our studies, we also observed increased UCP2 levels that might reduce ATP production, inducing compensatory hypertrophy. On the other hand, there were no significant changes in BiP and CHOP expression (not shown in the figure), indicating the absence of endoplasmic reticulum stress.

Fig. 6. Electron microscope images obtained from the myocardium: image from control diet rat with tissue section (parallel to fibers) (A), image from high-fat diet rat with the tissue section parallel to the muscle fibers (B), image from control diet rat with the tissue section taken perpendicular to the muscle fibers (C); and image from high-fat diet rat with the tissue section taken perpendicular to the muscle fibers (arrows show the extramyocellular lipids) (D).

The body weight gain in the high-fat diet group was associated with changes in cardiac function including increased heart rate, mild reduction in ejection fraction, increased left...
ventricular mass, reduced stroke volume, and increased cardiac output (Table 1). Insulin resistance affects cardiac function because of changes in lipid and glucose concentrations (45). The heart rate increase in high-fat diet rats was reported in earlier studies; the increased rate was sufficient to elevate cardiac output despite reduced ejection volumes (7). The stroke volume was reduced in high-fat diet rats by 5 to 6% compared with the controls. Earlier studies with rodents (7, 24) and humans (21) showed similar reductions in stroke volume associated with cardiac function. In our high-fat diet model with 20 wk of diet intervention, the changes in cardiac function were still within the normal range and did not yet exhibit cardiac dysfunction. However, the increased septal thickness supports the finding of hypertrophy, being consistent with the increased heart weight.

We observed two sets of lipid resonances in the $^1$H MRS spectra obtained from cardiac muscle of the high-fat diet rats. It is well known that in vivo MRS is sensitive to the geometry and type of tissue, and spectral features such as line shape and chemical shift are influenced by changes in the local magnetic field strength that arise from differences in the bulk magnetic susceptibility (BMS) between tissue (micro)compartments (4, 5, 38, 43). The BMS effects cause a change in Larmor frequencies (chemical shifts) of the same chemical species that are present in two different geometrical environments (“bodies”) (6). This finding is well established for skeletal muscle where the IMCL and EMCL pools have resonances that are separated by 0.2 ppm in $^1$H MRS spectra when the fibers are parallel to the magnetic field (38). EMCLs in long cylinders are nestled in the septa that are parallel to the muscle fiber bundles, whereas IMCLs are located within the cytoplasm as spherical droplets (18, 20). The high-resolution SEM images (Fig. 7), obtained from the location where the MRS was performed, showed the fibers to be predominantly parallel to the magnetic field direction. The $-\text{CH}_3$ signal from total creatine (phosphocreatine and creatine) showed a triplet centered at 3.03 ppm with the splitting being due to residual dipolar coupling; this outcome has been observed from skeletal muscle that is variously orientated relative to the direction of the magnetic field (4, 5, 31, 42–44). This independently confirmed that the fibers were predominantly parallel to the magnetic field. BMS effects on $^1$H MRS spectra were not known for cardiac muscle until a recent study demonstrated the presence of two lipid pools in $^1$H MRS of myocardium in the interventricular septum of patients with cardiac dystrophic mutations (34). In our study we demonstrated (for the first time to our knowledge) the presence of two lipid pools in the myocardium of rats fed a high-fat diet. We postulate that the separation of the $^1$H MR signals indicates the presence of two lipid pools that result from infiltration of fat between muscle fibers making a much larger EMCL pool than is normally present. It is well known that IMCL in skeletal muscle correlates with insulin resistance, diabetes, and obesity (5, 9). On the other hand, in
cardiac muscle we noticed that there was a significant increase in EMCL compared with IMCL. This might be due to high β-oxidative metabolism in the myocardium where there is rapid turnover of TGs compared with that in other organs, including liver and adipose tissues (22). Our TEM images (Fig. 6) are consistent with the 1H MRS findings (Fig. 4). The high-fat diet rats showed the presence of EMCL compared with placebo rats in myocardium, as shown in Fig. 6C.

We observed significant increase in heart TG levels (1.2-fold) in high-fat diet rats. It has been shown that increase in myocardial TGs precede the development of type 2 diabetes-induced cardiac dysfunction (26). The increased TG content in these rats might contribute to the increase in EMCL. The Oil Red O images showed evidence of larger accumulation of lipids in the high-fat diet rats. The data sampling errors were nevertheless large and can be judged to be only semiquantitative.

Conclusions. Our results showed that high-fat diet rats had moderate diabetes along with mild cardiac hypertrophy. We also report, for the first time, that two lipid pools can be identified by 1H MRS in the hearts of this rat model. Accumulation of fat between the muscle fibers where it is constrained to form elongated cylinders results in separate 1H MRS signals from TGs posits this as an early marker of lipid accumulation with mild cardiac hypertrophy associated with obesity and/or prediabetic conditions. Clinical studies could be done to evaluate whether cardiac dysfunction appears during the prediabetic phase or only after diabetes has been diagnosed. If the former is found, it could become the basis for deciding on earlier dietary, pharmaceutical, or surgical intervention. Rats receiving the high-fat diet showed significant increases in EMCL and heart weight. Further studies, for example with antiobesity interventions, will be required to explain the biochemical basis of the elevated EMCL in the myocardium.

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GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


Fig. 9. mRNA expression analysis: cluster of differentiation 36 (CD36; A), uncoupling protein 2 (UCP2; B), carnitine palmitoyltransferase (CPT1b; C), and medium chain acyl-CoA dehydrogenase (MCAD; D) for both control and high-fat diet rats. *P < 0.05, significant difference from control diet-fed group.
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