Abnormal in vivo myocardial energy substrate uptake in diet-induced type 2 diabetic cardiomyopathy in rats

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Ménard SL, Croteau E, Sarrhini O, Gélinas R, Brassard P, Ouellet R, Bentourkia M, van Lier JE, Rosiers CD, Lecomte R, Carpentier AC. Abnormal in vivo myocardial energy substrate uptake in diet-induced type 2 diabetic cardiomyopathy in rats. Am J Physiol Endocrinol Metab 298: E1049–E1057, 2010. First published February 16, 2010; doi:10.1152/ajpendo.00560.2009.—The purpose of this study was to determine in vivo myocardial energy metabolism and function in a nutritional model of type 2 diabetes. Wistar rats rendered insulin-resistant and mildly hyperglycemic, hyperinsulinemic, and hypertriglyceridemic with a high-fructose/high-fat diet over a 6-wk period with injection of a small dose of streptozotocin (HFHFS) and control rats were studied using micro-PET (μPET) without or with a euglycemic hyperinsulinemic clamp. During glucose clamp, myocardial metabolic rate of glucose measured with [18F]fluorodeoxyglucose ([18F]FDG) was reduced by ~81% (P < 0.05), whereas myocardial plasma nonesterified fatty acid (NEFA) uptake as determined by [18F]fluorothia-6-heptadecanoic acid ([18F]FTHA) was not significantly changed in HFHFS vs. control rats. Myocardial oxidative metabolism as assessed by [13C]acetate and myocardial perfusion index as assessed by [15N]ammonia were similar in both groups, whereas left ventricular ejection fraction as assessed by μPET was reduced by 26% in HFHFS rats (P < 0.05). Without glucose clamp, NEFA uptake was ~40% lower in HFHFS rats (P < 0.05). However, myocardial uptake of [18F]FTHA administered by gastric gavage was significantly higher in HFHFS rats (P < 0.05). These abnormalities were associated with reduced Glut4 mRNA expression and increased Cd36 mRNA expression and mitochondrial carnitine palmitoyltransferase 1 activity (P < 0.05). HFHFS rats display type 2 diabetes complicated by left ventricular contractile dysfunction with profound reduction in myocardial glucose utilization, activation of fatty acid metabolic pathways, and preserved myocardial oxidative metabolism, suggesting reduced myocardial metabolic efficiency. In this model, increased myocardial fatty acid exposure likely occurs from circulating triglyceride, but not from circulating plasma NEFA.

type 2 diabetes; heart failure; cardiomyopathy; energy metabolism; positron emission tomography; nonesterified fatty acids; glucose metabolism; triacylglycerol

Type 2 diabetes mellitus (T2D) is characterized by relative reduction of beta cell function with impaired glucose-stimulated insulin secretion in the face of insulin resistance in most tissues. Most patients with T2D have mild-to-moderate hyperglycemia (i.e., plasma glucose 10–30% above upper limit of normal) with mild increase in plasma triglyceride (TG) levels. These often asymptomatic and relatively subtle metabolic changes, nevertheless, lead to a major increase in micro- and macrovascular complications and to an important increase in incidence of cardiomyopathy and overt heart failure (4, 24, 30, 41). Although coronary artery disease and hypertension undoubtedly play a major role in the pathogenesis of T2D cardiomyopathy in humans, there is growing recognition that metabolic factors may play a role in its pathogenesis (23). Subclinical reduction in ventricular compliance and some degree of ventricular dilation have been recognized very early after onset of T2D in humans (54).

Some (3, 11, 28), but not all (31), studies have shown that energy production in the myocardium of humans and animals with T2D is more dependent on fatty acid oxidation at the expense of glucose utilization. Lipotoxicity is a major diabetogenic mechanism effective in animal models and humans alike and is characterized by reduction of glucose transport and utilization in response to excess tissue fatty acid exposure (16). Myocardial intramyocellular TG accumulation was recently demonstrated in subjects with glucose intolerance (33), and increased reliance on fatty acid oxidation may lead to reduced myocardial energy efficiency and may predispose to ischemic damage, intramyocardial accumulation of reactive lipids, and cardiomyocyte apoptosis (46, 47). Lipotoxicity has been implicated in the cardiomyopathy of several genetic animal models of T2D, such as the Zucker diabetic fatty (ZDF) rat (11, 42, 57). Genetic rodent models, however, often present very severe obesity and/or very severe hyperglycemia and dyslipidemia, to a degree rarely seen in most humans with T2D. Nutritional animal models of insulin resistance, such as high-fat- and/or high-carbohydrate-fed rats, also display mild systolic dysfunction with an increase in fatty acid utilization at the expense of glucose utilization (20, 32, 39). Previous studies have characterized the myocardial metabolic dysregulation of T2D cardiomyopathy in these nongenetic models using ex vivo heart perfusion models, but in vivo myocardial glucose, nonesterified fatty acid (NEFA), or global oxidative metabolisms were not reported in these models. The purpose of this study was to characterize in vivo myocardial substrate energy metabolism, myocardial perfusion, and ventricular function using micro-PET (μPET) methodologies in a rat model of diet-induced insulin resistance with relative beta cell dysfunction induced by injection of a small dose of...
streptozotocin to mimic metabolic abnormalities of T2D most often seen in humans.

MATERIALS AND METHODS

Animal experiments and diets. Eight-week-old male Wistar rats (Charles River, Quebec, Canada) were housed with a 12:12-h light-dark cycle at constant temperature (22°C). The control (CON) group was fed ad libitum regular chow (Rodent Laboratory Chow 5001, Purina, St. Louis, MO), and the T2D high-fat-, high-fructose-fed rats injected with a small dose of streptozotocin (HHFHS) were fed a high-fat–high-fructose diet (TD 05482 rat chow, Teklad) containing 46.5 wt% fructose and 25.7 wt% lard for 6 wk; both groups had free access to water. At 2 wk after initiation of the diet, HHFHS rats were injected intraperitoneally with a small dose of streptozotocin (25 mg/kg) and CON rats were injected with vehicle. All experiments were performed after a 12-h fasting period under anesthesia with 2.0% (vol/vol) isoflurane (Abbott Laboratories, Montreal, Canada) delivered through a nose cone. Catheters were placed into a carotid artery (vol/vol) and were kept open with infusion of 0.9% saline. All animal protocols were approved by the Animal Ethics Committee of the Faculty of Medicine of the Université de Sherbrooke in accordance with the guidelines of the Canadian Council on Animal Care.

Small animal PET protocol. All in vivo experiments (Fig. 1) were initiated 60 min after insertion of catheters and performed under normal fasting or euglycemic hyperinsulinemic conditions, as previously described (17). At time 0 of the glucose clamp experiments, a primed (180 mU/kg) constant insulin infusion (12 mU·kg⁻¹·min⁻¹ in 0.1% BSA in physiological saline; Novolin GE, Toronto, Canada) was started and continued for 2 h to standardize the metabolic condition in all in vivo protocols. Whole blood glucose level was maintained using a variable 20% dextrose intravenous infusion according to the glucose level, determined every 10 min using a blood glucose monitor (Accusoft Advantage, Roche) (17).

Imaging experiments were performed with the avalanche photodiode-based small animal PET scanner (μPET) of the Sherbrooke Molecular Imaging Centre (29). Before imaging, the heart position was localized with a Doppler probe (0.64 cm, 9 MHz; Parks Medical Electronics). During imaging, the animals rested supine on the scanner bed and were kept warm with a heating pad. Boluses of each radiopharmaceutical (30–40 MBq, in 0.5 ml of 0.9% NaCl) were injected via the caudal vein over 30 s. In one set of experiments, a 10-min dynamic acquisition with [¹⁸F]acetate and a 30-min dynamic acquisition with [¹⁸F]fluorodeoxyglucose ([¹⁸F]FDG) were done to determine myocardial oxidative metabolism ([O₂ uptake (VO₂)]) and glucose utilization (myocardial metabolic rate of glucose (MMRG)), respectively, as previously described (35). In another set of experiments, a 10-min dynamic acquisition with [¹¹C]ammonia and a 30-min dynamic acquisition with [¹⁸F]fluorothio-6-heptadecanoic acid (FTHA) were used to determine myocardial blood flow and NEFA uptake ([¹¹C]acetate and [¹⁸F]FTHA acquisition. Blood samples were taken at baseline and at 90, 100, 110, and 120 min to determine blood glucose and plasma insulin and NEFA levels (35).

Additional experiments were performed to assess myocardial fatty acid uptake from circulating triacylglycerols (TGs) with administration of [¹⁸F]FTHA and [¹³N]ammonia (~30 MBq and ~7.5 μCi, respectively, in 0.5 ml of 20% Intralipid) followed by blood sampling over 2 h and assessment of myocardial tracer uptake.

Imaging data analysis. For [¹¹C]ammonia and [¹⁸F]acetate images, dynamic series of 23 frames (1 for 30 s, 12 for 5 s, 8 for 30 s, and 2 for 100 s) each, were sorted out, whereas 26 frames (1 for 30 s, 12 for 5 s, 8 for 30 s, and 5 for 300 s) were used for [¹⁸F]FDG and [¹⁸F]FTHA imaging and were reconstructed as a series of adjacent two-dimensional slices using 15 iterations of the maximum-likelihood expectation maximization algorithm. Regions of interest (ROIs) were drawn on short-axis images. MMRG and VO₂ were quantified in matching ROIs, while Kₐ and myocardial blood flow were matching together. Input curves were extracted by means of an ROI drawn on the left ventricular cavity blood pool in summed last-frame images to seek better contrast. The sizes of these almost-circular ROIs were compared with images of eight cylinders of different diameters from which a recovery factor was extracted and applied to the ROIs for partial volume correction (43). Furthermore, the images were corrected for the count contamination (spillover) from tissue to blood by means of factor analysis of dynamic structures, which allowed isolation of blood and tissue images (2, 7).

For [¹⁸F]acetate, we used a three-compartment kinetic model that estimates the generation of CO₂ from the citric acid cycle in the myocardium using the k₂ value (6). We used a three-compartment kinetic model for [¹¹C]ammonia that provides an estimate of blood flow from the k₁ value (18). The fractional myocardial glucose uptake (MGup) and MMRG were determined by multicompartmental analysis of the [¹⁸F]FDG data (35, 40). MGup was derived from the three glucose kinetic constants, Kₐ, k₂, and k₃ [e.g., Kₐ·k₂/(k₃ + k₂)], a method that is analogous to linearization modeling techniques previously used for determination of myocardial glucose metabolism in rats (44). MMRG is calculated using the following equation

\[ LC \cdot MGup \cdot \text{blood glucose level} \]

where LC is the lumped constant with a value of 1 (44).

Fig. 1. Experimental protocols. μPET, micro-PET; dyn, list-mode dynamic acquisition; FDG, fluorodeoxyglucose; FTHA, fluorothio-6-heptadecanoic acid; gat, ECG-gated dynamic acquisition mode.
The nonmetabolized fraction of $[^{18}F]$FTHA in plasma was determined using thin-layer chromatography from blood samples taken at 1, 2, 3, 5, 10, 20, and 30 min after $[^{18}F]$FTHA injection (Fig. 2A), and the metabolite-corrected input curve (Fig. 2B) was calculated by linear interpolation and used to correct the plasma input function (17). Myocardial NEFA fractional uptake ($K_i$) and $K_m$ were determined by a Patlak graphical analysis (35, 40).

\[
\frac{\text{ROI}(t)}{C_p(t)} = \frac{K_i}{k_2} + \frac{K_i}{(k_2 + k_3)} \times \left( \frac{C_p(t)\,d(t)}{C_p(t)} \right)
\]

where ROI$(t)$ is $[^{18}F]$FTHA tissue ROI activity at time $t$, $C_p(t)$ is the $[^{18}F]$FTHA plasma activity at time $t$, and $\int C_p(t)\,d(t)$ is the integrated plasma $[^{18}F]$FTHA activity up to time $t$ corrected for the presence of plasma $[^{18}F]$FTHA metabolites. The relation between ROI$(t)/C_p(t)$ and $[C_p(t)\cdot d(t)]/C_p(t)$ is linear for $[^{18}F]$FTHA in the myocardium, and the term $(K_i/k_2)/(k_2 + k_3) = K_s$ is the steady-state trapping rate of the tracer. Thus the slope ($K_i$) of this relationship represents tissue NEFA fractional uptake (in ml·g⁻¹·min⁻¹). Therefore

\[
\text{uptake}_{\text{NEFA}}(K_m) = K_i \times \left( \frac{\text{NEFA}}{\text{LC}} \right)
\]

where LC is the lumped constant for $[^{18}F]$FTHA compared with endogenous plasma NEFA (i.e., 1.0) and NEFA is the steady-state plasma NEFA concentration.

For analysis of ventricular function, PET data from $[^{18}F]$FTHA images were obtained as a series of eight ECG-gated frames and were reconstructed as a series of adjacent two-dimensional slices using 20 iterations of the maximum-likelihood expectation maximization algorithm. Corridor4DM version 5.2 software (Segami, Invia) was used for reorientation and to compute left ventricular volumes and left ventricular ejection fraction (LVEF) after validation with small rodent heart phantoms, as described previously (19).

**Plasma and tissue assays.** Plasma insulin, NEFA, and TG levels were measured as previously described (35). Total plasma adiponectin was determined by ELISA (Alpco Diagnostics, Salem, NH). Plasma and tissue lipids were extracted according to the method described by Folch et al. (22). Myocardial fibrosis was quantified using histochemistry for collagen type I fibers on paraffin-embedded tissue sections stained with Sirius red F3BA and analyzed by cross-polarized microscopy, as described elsewhere (13). Myocardial mitochondria were extracted and glutamate dehydrogenase activity was measured to correct for extraction efficiency (17). Mitochondrial carnitine palmitoyltransferase-I (CPT-I) activity was assayed using the method of Drynan et al. (21). Briefly, mitochondrial extracts (0.5 mg of proteins) were incubated at 37°C in 150 mM KCl, 1 mM EGTA, 5 mM Tris–HCl (pH 7.4), 5 mM ATP, 1% (wt/vol) BSA, 1.6 mM dithiothreitol, 4 μg/ml rotenone, 2 μg/ml antimycin A, and 0.52 mM L-[3H]carnitine (770 dpm/nmol) in the presence of 0–100 μM malonyl-CoA. The reactions were stopped by addition of 0.3 ml of 6 M HCl, and [3H]palmitoylcarnitine was extracted in 1 ml of n-butanol and its content was determined by scintillation counting (Coulter, Beckman).

Reverse transcription quantitative polymerase chain reaction gene expression analysis. HFHFS and CON rats were killed in fasting and in nonfasting states at 8 AM. Hearts were flash frozen in liquid nitrogen, and mRNA was extracted to determine the myocardial relative mRNA levels of selected genes related to fatty acid [medium-chain acyl-CoA dehydrogenase (Acadm, McaD); Pdk4, pyruvate dehydrogenase kinase 4 (Pdk4); Pydh4, pyruvate dehydrogenase kinase 4 (Pdk4); ATPcit, adenosine triphosphatase citrate (ATPCit); Cpt1b, carnitine palmitoyltransferase-1 (CPT-1) activity was assayed using the method of Folch et al. (22). Myocardial fibrosis was quantified using histochemistry for collagen type I fibers on paraffin-embedded tissue sections stained with Sirius red F3BA and analyzed by cross-polarized microscopy, as described elsewhere (13). Myocardial mitochondria were extracted and glutamate dehydrogenase activity was measured to correct for extraction efficiency (17). Mitochondrial carnitine palmitoyltransferase-I (CPT-I) activity was assayed using the method of Drynan et al. (21). Briefly, mitochondrial extracts (0.5 mg of proteins) were incubated at 37°C in 150 mM KCl, 1 mM EGTA, 5 mM Tris–HCl (pH 7.4), 5 mM ATP, 1% (wt/vol) BSA, 1.6 mM dithiothreitol, 4 μg/ml rotenone, 2 μg/ml antimycin A, and 0.52 mM L-[3H]carnitine (770 dpm/nmol) in the presence of 0–100 μM malonyl-CoA. The reactions were stopped by addition of 0.3 ml of 6 M HCl, and [3H]palmitoylcarnitine was extracted in 1 ml of n-butanol and its content was determined by scintillation counting (Coulter, Beckman).

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### Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>CON (n = 44)</th>
<th>HFHFS (n = 48)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total caloric intake, kcal/6 wk</td>
<td>3,808 ± 58</td>
<td>4,049 ± 71</td>
<td>0.02</td>
</tr>
<tr>
<td>Total water intake, ml/6 wk</td>
<td>1,897 ± 75</td>
<td>1,669 ± 49</td>
<td>0.03</td>
</tr>
<tr>
<td>Final body wt, g</td>
<td>435 ± 8</td>
<td>448 ± 4</td>
<td>0.09</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>7.1 ± 0.2</td>
<td>10.1 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>302 ± 39</td>
<td>498 ± 42</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Fasting NEFA, μmol/l</td>
<td>959 ± 57</td>
<td>786 ± 42</td>
<td>0.07</td>
</tr>
<tr>
<td>Fasting TG, μmol/l</td>
<td>502 ± 40</td>
<td>775 ± 92</td>
<td>0.03</td>
</tr>
<tr>
<td>Fasting adiponectin, μg/l</td>
<td>1,535 ± 56</td>
<td>1,255 ± 350</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. CON, control; HFHFS, high-fat, high-fructose-fed rats treated with streptozotocin; NEFA, nonesterified fatty acids; TG, triglyceride. *Unpaired Student’s t-test; $t_n = 27$ and 31 in CON and HFHFS, respectively; $t_n = 16$ and 24 in CON and HFHFS, respectively.
Table 2. Euglycemic hyperinsulinemic clamp data

<table>
<thead>
<tr>
<th>Group</th>
<th>CON (n = 20)</th>
<th>HFHFS (n = 22)</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>5.5 ± 0.1</td>
<td>5.3 ± 0.3</td>
<td>0.12</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>4.407 ± 558</td>
<td>6.991 ± 623</td>
<td>0.001</td>
</tr>
<tr>
<td>NEFA, μmol/l</td>
<td>656 ± 27</td>
<td>707 ± 24</td>
<td>0.33</td>
</tr>
<tr>
<td>TG, μmol/l</td>
<td>435 ± 74</td>
<td>625 ± 98</td>
<td>0.01</td>
</tr>
<tr>
<td>Ginf, μmol/min</td>
<td>42.2 ± 1.7</td>
<td>21.9 ± 1.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ginf, glucose infusion rate. *Unpaired Student’s t-test.

**Statistical analysis. Values are means ± SE. Unpaired Student’s t-test, Mann-Whitney test, or two-way ANOVA was performed when appropriate using GraphPad Prism version 5.00 for Windows (San Diego, CA). Two-tailed P < 0.05 was considered significant.

**RESULTS**

**HFHFS** rats have normal weight and are insulin-resistant. Table 1 shows the characteristics of HFHFS and CON rats. Calorie and water intake and weight gain were similar in both groups over the 6-wk feeding period. Final body weight was also similar, whereas fasting blood glucose and plasma insulin and TG levels were significantly higher and plasma total adiponectin was significantly lower in HFHFS than CON rats at the end of the 6-wk feeding period. NEFA also tended to be lower in HFHFS rats (P = 0.07). Table 2 shows results from euglycemic hyperinsulinemic clamps during the imaging protocols after an overnight fast in HFHFS and CON rats. By design, blood glucose level was similar in the two groups during the clamp. As expected, despite higher plasma insulin levels, glucose infusion rates (Ginf) were ~50% lower in HFHFS than CON rats. During the clamp, plasma TGs were significantly higher in the HFHFS than CON rats, but plasma NEFA levels were similar in the HFHFS and CON groups.

**HFHFS** rats have altered in vivo myocardial energy substrate metabolism but normal myocardial blood flow. During euglycemic hyperinsulinemic clamp, MGup and MMRG assessed by μPET after intravenous injection of [18F]FDG were profoundly (~81%) reduced in HFHFS compared with CON rats (Fig. 3, A–C; P < 0.001), whereas fractional uptake of [18F]FTHA (Ki) and myocardial NEFA uptake (Km) (Fig. 3, D–F) were not significantly changed (P = 0.33 and P = 0.28, respectively). During fasting without glucose clamp, MGup was too low in both groups to allow accurate determination of MMRG with [18F]FDG (data not shown). In this condition, [18F]FTHA Ki was similar in both groups (Fig. 3G), whereas [18F]FTHA Km was significantly lower in HFHFS than CON rats (P < 0.05; Fig. 3H). In addition, myocardial oxidative metabolism (V̇O2) assessed using the [13N]ammonia Ki kinetic parameter was unchanged in HFHFS vs. CON rats. HFHFS rats also had similar myocardial perfusion index compared with CON rats as assessed by the [13N]ammonia Ki kinetic parameter (Fig. 4B).

**HFHFS** rats have increased in vivo myocardial fatty acid uptake from circulating TG. After gastric gavage, plasma glucose, insulin, and TG were higher in HFHFS than CON rats (all P < 0.05) and plasma NEFA levels were similar in HFHFS and CON rats (not shown). Postgavage plasma [18F]FTHA and [3H]triolein activity were similar in both groups (Fig. 5, A and B), but
myocardial [18F]FTHA activity was higher in HFHFS than CON rats (Fig. 5C; P < 0.05).

Activation of myocardial lipid metabolic pathways in HFHFS rats. Cardiac levels of mRNA encoding for Cd36 (Fig. 6A), but not Acadm (not shown), were increased in the fasting and fed conditions in HFHFS vs. CON rats. CPT-1 activity of myocardial mitochondrial extracts was significantly higher and more resistant to inhibition by malonyl-CoA in HFHFS than CON rats (Fig. 6B and C). We also found that myocardial Glut4 mRNA level in the nonfasting state was lower in HFHFS than CON rats (Fig. 6D), whereas no significant difference in Pfkm mRNA levels was found (not shown). Myocardial Pdk4 mRNA expression was lower in the nonfasting than fasting condition but was similar in HFHFS and CON rats (not shown).

HFHFS rats display left ventricular systolic dysfunction. Table 3 shows heart rate, blood pressure, rate-pressure product, and left ventricular volumes and LVEF assessed by µPET after injection of [18F]FTHA in HFHFS and CON rats. Heart rate, mean blood pressure, and rate-pressure product were similar in both groups during the studies. End-diastolic volume was similar, but end-systolic volume was significantly higher in HFHFS than CON rats. LVEF was reduced 26% in the HFHFS group. Mean heart weight and heart-weight-to-body weight ratio were, however, similar in HFHFS and CON rats (not shown), and no significant increase in cardiac fibrosis was observed by histochemistry for collagen fiber type I (Fig. 7).

DISCUSSION

We found that 6 wk of high-fat–high-fructose feeding with injection of a small dose of streptozotocin in rats results in mild hyperglycemia and hypertriglyceridemia associated with mild fasting hyperinsulinemia, hyperadiponectinemia, and whole body insulin resistance, as determined by euglycemic hyperinsulinemic clamp, without significant increase in body weight. These metabolic perturbations, reminiscent of T2D in humans, were associated with profound reduction in myocardial glucose utilization but without significant change in myocardial NEFA uptake, myocardial perfusion, and myocardial oxidative metabolism during glucose clamp. Both plasma NEFA level and myocardial NEFA uptake were even reduced during fasting without glucose clamp in HFHFS rats. Myocardial fatty acid uptake from circulating TG, however, was increased in our rat

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Fig. 4. Myocardial oxidative metabolism determined by µPET after intravenous injection of [11C]acetate (k2, A) and myocardial perfusion index determined by µPET after intravenous injection of [13N]ammonia (k1, B) in HFHFS (n = 6 for [11C]acetate and n = 8 for [13N]ammonia) and CON (n = 7 for [11C]acetate and n = 8 for [13N]ammonia) rats.

Fig. 5. Plasma [3H]triolein (A) and [18F]FTHA (B) activity after gastric gavage in HFHFS (n = 4, ●) and CON (n = 5, ○) rats. C: myocardial [18F]FTHA uptake 2 h after administration by gastric gavage in HFHFS (n = 4) and CON (n = 3) rats. %ID, percent injected dose.
model of T2D. Reduced in vivo myocardial glucose utilization was associated with albeit less striking but consistent modifications of cardiac metabolic pathways at the molecular level, including reduction in feeding-stimulated Glut4 mRNA expression, and with activation of myocardial fatty acid metabolic pathways, including increased Cd36 mRNA expression and increased CPT-1 activity. Finally, significant reduction in LVEF with reduced stroke volume and similar heart rate without development of cardiac fibrosis was observed in HFHFS rats.

To our knowledge, this is the first study to characterize cardiac energy metabolism in a nutritional animal model of T2D cardiomyopathy using in vivo PET metabolic imaging techniques. Major advances in knowledge of cardiac function and metabolism of type 1 and type 2 diabetic cardiomyopathy stemmed from ex vivo isolated perfusion systems without or with study of in vivo cardiac function by echocardiography (see Refs. 11, 42, and 28 for review). Most of these models display some degree of systolic and/or diastolic cardiac dysfunction with cardiac hypertrophy but without fibrosis. Most of these studies have been performed using genetic models of T2D cardiomyopathy, which tend to display a marked degree of hyperglycemia, hyperinsulinemia, and hyperlipidemia, with markedly increased myocardial lipid content and no change in cardiac perfusion. Although these methods are essential for advance in this field of research, one important word of caution regarding ex vivo isolated heart perfusion studies is that these conditions do not recapitulate in vivo availability of energy substrates other than glucose and NEFA (e.g., lactate, pyruvate, ketone bodies, and TGs) and that substrate levels (e.g., concentrations of glucose, insulin, and fatty acids) selected may affect cardiac metabolism and function in these studies (12, 28).

Several pathophysiological mechanisms may contribute to the development of diabetic cardiomyopathy, including altered cardiac calcium homeostasis, activation of the renin-angiotensin system, increased oxidative stress, mitochondrial uncoupling and dysfunction, and altered myocardial substrate metabolism (see Ref. 11 for review). Ex vivo isolated heart perfusion experiments have clearly shown reduced myocardial glucose and lactate utilization with increased utilization of fatty acids often associated with intramyocardial TG accumulation in the insulin-resistant and diabetic heart (11). Reduced myocardial glucose utilization in humans with T2D has been documented in some (38, 52, 56), but not all (31), studies using PET,
whereas a small absolute increase of intramyocardial TG content has been recently supported by proton magnetic resonance spectroscopy in prediabetic and diabetic humans (26, 34, 46). Fatty acids may impair insulin signaling and may activate several signaling pathways implicated in cardiac oxidative metabolism, including peroxisome proliferator-activated receptor-α (PPARα) (11, 16). Increased uptake of plasma NEFA likely contributes to increased fatty acid utilization at the expense of reduced glucose oxidation and to the development of cardiac lipotoxicity and diabetic cardiomyopathy in genetic rodent models of T2D (28). Indeed, a previous study in ZDF rats found a significant increase in myocardial plasma NEFA utilization and oxidation determined in vivo by PET driven by the grossly elevated (>2-fold) plasma NEFA levels in this model (53). Another recent study in ZDF rats also found a 66% reduction in myocardial glucose uptake associated with a 41% increase in myocardial NEFA uptake using μPET (51). It is notable, however, that previous human studies using PET (27, 50) and another in db/db mice using in vivo radioactive tracer methods (37) failed to demonstrate a significant increase in myocardial NEFA uptake associated with T2D, more in line with our findings. It should be noted that elevation of plasma NEFA levels in subjects with insulin resistance and T2D is often not significant unless fat loading is performed (14, 15). The higher insulin concentration in the HFHFS group may have contributed to the relatively low plasma NEFA levels and myocardial NEFA uptake in HFHFS rats by suppression of adipose tissue lipolysis. In humans with T2D, plasma NEFA levels and appearance rate during fasting conditions, especially with adrenergic stimulation, were normal or even reduced in many studies (8, 9, 36). This probably reflects the lower adipose tissue lipolytic capacity in insulin-resistant states (25). It is also possible that higher plasma glucose may have contributed to relative reduction of myocardial NEFA uptake during fasting conditions without normalization of plasma glucose with insulin infusion in the present study (45). Therefore, changes in plasma NEFA levels in different physiological states can probably explain some of the discrepancies between studies and models.

The demonstration of increased Cd36 mRNA expression with enhanced CPT-1 activity and reduced suppression by malonyl-CoA, perhaps secondary to PPARα activation (11), provides molecular mechanisms for preferential selection of fatty acids to support mitochondrial energy production in diabetic cardiomyopathy, consistent with findings in other animal models of insulin resistance and diabetes (10, 37, 39, 51). The absence of change in Pdk4 expression, however, does not support PPARα-mediated inhibition of glucose oxidation with diabetic cardiomyopathy in the present study (11). One limitation of the present study is that we did not assess directly cardiac transmembrane transport of fatty acids and glucose, nor did we assess the expression level of fatty acid and glucose transport proteins. Using giant vesicle preparations from cardiac cells in ZDF rats, other investigators showed a significant increase in transmembrane fatty acid transport and CD36 plasma membrane expression (10). These ex vivo experiments, however, do not account for changes in plasma NEFA levels in the different models of T2D, a critical factor driving myocardial fatty acid uptake in the in vivo setting.

The similar rate of myocardial oxidative metabolism, despite reduced stroke volume, in the present study is also consistent with reduced efficiency of myocardial energy production associated with the relative increase in reliance on fatty acid oxidation at the expense of glucose oxidation in the diabetic heart (reviewed in Ref. 11). Another source of fatty acids, such as circulating TG, may significantly contribute to myocardial oxidative metabolism and lipotoxicity in T2D (5, 49). Our experiments showing increased myocardial uptake of orally administered [18F]FTHA that is delivered to the tissues through circulating TG support this possibility. The plausibility of this hypothesis is also supported by the development of a lipotoxic cardiomyopathy in mice with transgenic cardiac lipoprotein lipase expression (55). We recently found that hypertriglyceridemia acutely reduces myocardial glucose utilization and myocardial perfusion in rats (35), suggesting a role for hypertriglyceridemia as well to explain these modifications in HFHFS rat myocardium. Fenofibrate treatment was shown to improve cardiac function and metabolism in diet-induced obese mice through improvement of hepatic lipid metabolism and reduced circulating lipids (1).

In humans, myocardial NEFA uptake determined using [18F]FTHA was similar to that determined using [11C]palmitate (27, 50). Another limitation of our study, however, is that myocardial glucose and NEFA oxidation rates cannot be directly assessed using [18F]FDG and [18F]FTHA. However, >90% of myocardial 18F activity after intravenous injection of FTHA is retrieved in mitochondria (17, 48), suggesting that myocardial FTHA uptake is a good marker of myocardial NEFA oxidation.

In conclusion, HFHFS rats, an animal model of T2D with mild hyperglycemia, hyperinsulinemia, insulin resistance, hypertriglyceridemia, and hyperadiponectinemia, display a cardiomyopathy characterized by profound reduction in myocardial glucose utilization and activation of myocardial fatty acid

![Image](https://via.placeholder.com/150.png?text=Image+1)

Fig. 7. Sirius red F3BA ventricular staining showing no difference in collagen type I fiber content by cross-polarized microscopy between CON (A) and HFHFS (B) rats. Original magnification ×40.
oxidation molecular pathways, but without a significant increase in myocardial NEFA uptake, total oxidative metabolism, and myocardial perfusion in vivo. Preliminary evidence suggests that myocardial fatty acid uptake from circulating TG may be increased in our model of T2D. This nutritional model of mild T2D is thus an interesting model of metabolic cardiomyopathy with altered myocardial substrate metabolism and impaired efficiency of myocardial energy production.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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