Cardiac Steatosis in Diabetes Mellitus
A ¹H-Magnetic Resonance Spectroscopy Study

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Background—The risk of heart failure in type 2 diabetes mellitus is greater than can be accounted for by hypertension and coronary artery disease. Rodent studies indicate that in obesity and type 2 diabetes mellitus, lipid overstorage in cardiac myocytes produces lipotoxic intermediates that cause apoptosis, which leads to heart failure. In humans with diabetes mellitus, cardiac steatosis previously has been demonstrated in explanted hearts of patients with end-stage nonischemic cardiomyopathy. Whether cardiac steatosis precedes the onset of cardiomyopathy in individuals with impaired glucose tolerance or in patients with type 2 diabetes mellitus is unknown.

Methods and Results—To represent the progressive stages in the natural history of type 2 diabetes mellitus, we stratified 134 individuals (age 45±12 years) into 1 of 4 groups: (1) lean normoglycemic (lean), (2) overweight and obese normoglycemic (obese), (3) impaired glucose tolerance, and (4) type 2 diabetes mellitus. Localized ¹H magnetic resonance spectroscopy and cardiac magnetic resonance imaging were used to quantify myocardial triglyceride content and left ventricular function, respectively. Compared with lean subjects, myocardial triglyceride content was 2.3-fold higher in those with impaired glucose tolerance and 2.1-fold higher in those with type 2 diabetes mellitus (P<0.05). Left ventricular ejection fraction was normal and comparable across all groups.

Conclusions—In humans, impaired glucose tolerance is accompanied by cardiac steatosis, which precedes the onset of type 2 diabetes mellitus and left ventricular systolic dysfunction. Thus, lipid overstorage in human cardiac myocytes is an early manifestation in the pathogenesis of type 2 diabetes mellitus and is evident in the absence of heart failure.

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Key Words: magnetic resonance spectroscopy ■ obesity ■ metabolism

The obesity epidemic has led to a parallel rise in the prevalence of type 2 diabetes mellitus (T2D), with its attendant risk of cardiovascular disability and death.¹² The risk for heart failure in T2D is greater than can be accounted for by traditional antecedent factors such as hypertension and coronary artery disease.³ Altered substrate metabolism is thought to contribute importantly to dysfunction of the diabetic heart.⁴–⁸ In obesity and T2D, the contribution of glucose oxidation to cardiac energetics is less than normal, and the reliance on fatty acid metabolism is enhanced.⁹

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Exactly how such metabolic derangements lead to cardiac dysfunction is unknown, but one increasingly popular theory involves lipid overstorage and lipotoxic injury to cardiac myocytes.¹⁰–¹⁴ In the setting of excessive free fatty acid delivery associated with obesity and T2D, fatty acid uptake by cardiac myocytes likely exceeds mitochondrial oxidative capacity, which may be additionally impaired by several putative mechanisms (including increased expression of uncoupling proteins by reactive oxygen species and lipid peroxidation products).⁹ The resultant lipid overstorage (ie, cardiac steatosis) produces lipotoxic intermediates such as ceramide that increase production of reactive oxygen species and cause apoptosis.¹¹

These mechanistic conclusions have been derived mainly from rodent models,¹⁵–¹⁹ with translational human research being far less developed. One previous human study using oil red O staining of explanted hearts at the time of cardiac transplantation demonstrated cardiac steatosis in diabetic patients with heart failure.¹⁴ Whether cardiac steatosis is the cause or the consequence of the heart failure is unknown. Using in vivo magnetic resonance spectroscopy (MRS), here, we demonstrate cardiac steatosis in individuals with impaired glucose tolerance (IGT) and in patients with T2D.
Methods

Subjects
We recruited 177 participants for the present study. Cardiac spectra from 43 participants were distorted by motion and were excluded from analysis. The remaining 134 participants completed all study procedures and were included in the final analysis.

The Institutional Review Board at the University of Texas Southwestern Medical Center approved all experimental protocols, and all participants provided written informed consent before their participation in the study, according to the guidelines established in the Declaration of Helsinki. Nondiabetic subjects were recruited from the Dallas Heart Study database, a population-based random sample of Dallas County, Tex, residents who agreed to have multiple assessments of risk factors for cardiovascular disease, including cardiac imaging.20 Subjects with T2D were recruited from the Diabetes Clinic at Parkland Memorial Hospital in Dallas. Nondiabetic subjects were seen in the outpatient General Clinical Research Center in a fasted state to determine glucose tolerance. All subjects had height, weight, and blood pressure measured before magnetic resonance imaging and spectroscopy.

To represent the progressive stages in the natural history of T2D, subjects were stratified into 4 groups based on body mass index (BMI) and/or blood glucose at the end of a 2-hour oral glucose (75 g) tolerance test: (1) lean (BMI <25 kg/m²) normoglycemic (2-hour glucose <140 mg/dL); (2) overweight and obese (BMI ≥25 kg/m²) normoglycemic; (3) IGT (2-hour glucose 140 to 199 mg/dL); and (4) T2D (history of diabetes mellitus or 2-hour glucose ≥200 mg/dL). Exclusion criteria were as follows: (1) age >70 years, (2) known presence of or medical treatment for coronary artery disease, (3) previous myocardial infarction, (4) metallic implants, (5) pregnancy, or (6) claustrophobia. Additionally, subjects with T2D were excluded if they were treated with thiazolidinediones, because these drugs are known to alter intracellular lipid content.21

Patients with diabetes mellitus were asked to stop taking all oral hypoglycemic agents and were initiated on or had intensification of their insulin treatment for 2 weeks before the evaluation. The preenrollment treatment regimen of all the patients was as follows: glyburide (29%), glyburide and insulin (9%), glyburide and metformin (12%), metformin and insulin (21%), insulin only (17%), and no medications (12%).

Myocardial Imaging and Spectroscopy
Localized spectroscopy can distinguish between triglyceride droplets localized in the cytosol of cardiomyocytes (ie, an aqueous microenvironment) and triglycerides stored in adipocytes (ie, a lipid microenvironment). During 1H-MRS, these different microenvironments cause triglycerides to resonate at different frequencies. Triglyceride droplets within cytosol resonate at 1.4 ppm, and triglycerides located in adipocytes resonate at 1.6 ppm relative to resonance from tissue water at 4.8 ppm.10 The validation and reproducibility of the method have been published previously.10,21,22 The experimental setup for the measurement of myocardial triglyceride content is provided in Figure 1.

To determine left ventricular (LV) morphology, function, and triglyceride content, we used a 1.5-Tesla Gyroscan INTERA whole-body system (Philips Medical Systems, Best, The Netherlands) equipped with spectroscopy and cardiac packages as described previously.21,22 After anatomic imaging, we positioned the spectroscopic volume of interest (6 cc to 10×30 mm3) within the interventricular septum using the end-systolic cardiac cine images in 3 planes, collected as patients held their breath at end expiration.21 During acquisition of spectroscopic data, patients breathed freely. The spectroscopic signal was acquired with cardiac triggering at end systole and respiratory gating at end expiration. We used the PRESS sequence (Point-RESolved Spectroscopy) for spatial localization, and the interpulse delay was defined by the length of a respiratory cycle (~4 seconds). NUTS software (Acorn NMR, Fremont, Calif) was used to process data. The areas under the signals from water and methylenes of fatty acids in triglycerides were quantified by a line-fitting procedure, and the values were corrected for spin-spin relaxation.21,22 The myocardial triglyceride content was expressed as a ratio of fat to water (%).

Dynamic cine images were used to quantify LV volume.23,24 Image analysis was performed by an observer blinded to the subject’s clinical history using a commercially available workstation (MASS, Philips Medical Systems). Endocardial and epicardial LV borders were traced manually at end diastole and end systole from short-axis slices, and the papillary muscles were excluded from the LV cavity volume.

LV mass was computed as the product of end-diastolic LV volume and myocardial density (1.05 g/mL).25 Ejection fraction was used as an index of global LV function.25 LV diastolic function was determined by the conventional multislice volumetric technique as reported previously.26–28 LV blood volume was calculated for every phase of the cardiac cycle. LV diastolic filling was determined by measuring the peak rate of change in LV volume during the first one third of diastole, before atrial contraction. This magnetic resonance imaging (MRI) measurement of early diastolic peak filling has been validated in the past.29 Magnetic resonance computation of diastolic dysfunction is based on a direct measurement of LV volume and thus constitutes a more reliable index of ventricular filling rate than estimates derived from echocardiography and Doppler velocimetry.26–28

Metabolic Parameters
Hepatic triglyceride levels were determined by 1H-MRS as described previously.29,30 Subcutaneous and visceral abdominal fat masses were determined from abdominal axial images at the L2 to L3 level.31

Assays to determine serum concentrations of glucose, plasma lipoproteins, serum triglycerides, and liver enzymes were performed on a Beckman CX9ALX chemical analyzer (Beckman-Coulter, Fullerton, Calif). Plasma nonesterified fatty acids and insulin concentrations were assayed as described previously.32,33

Statistical Analyses
Differences in participant characteristics, metabolic variables, and hemodynamics across the 4 groups were tested with ANOVA models with Bonferroni post hoc correction for normally distributed variables and Kruskal-Wallis tests for nonnormally distributed variables. χ2 Tests were used to detect differences in the distribution of gender and ethnicity between the groups. ANCOVA models included age, BMI, serum triglyceride, and gender as covariates to statistically control for baseline differences in these variables. Stepwise multiple regression analysis was used to determine whether group (ie, lean, obese, IGT, and T2D), age, body mass index, or serum lipids were independently related to myocardial triglyceride. Pearson r was used.
for bivariate correlation analysis. SPSS software (version 14.0; SPSS Inc, Chicago, Ill) was used for the primary statistical analyses. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

The characteristics and metabolic variables of study participants are summarized in Table 1. Normoglycemic individuals were on average 10 years younger than individuals with IGT and T2D (P<0.01). The average BMI was 23 kg/m² in the lean group and in the obese range (>30 kg/m²) in the remaining 3 groups. Fasting glucose, insulin, and the homeostasis model assessment index were higher in T2D than in the remaining 3 groups. Fasting triglycerides were within normal limits in the lean group and were elevated 2-fold in the remaining groups (P<0.01). Fasting triglycerides curve (FFA AUC) during the 2-hour oral glucose tolerance test was higher in the IGT and T2D groups than in the normoglycemic groups (P<0.01). Myocardial triglyceride content was elevated in the obese group and was higher in the obese and T2D groups than in the lean group (P=0.02). The average duration of diabetes mellitus from the time of diagnosis was 5.0±6.2 years.

Table 2. Participant Hemodynamics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lean (n=15)</th>
<th>Obese (n=21)</th>
<th>IGT (n=20)</th>
<th>T2D (n=78)</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>122±15</td>
<td>123±13</td>
<td>129±18</td>
<td>136±18†</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>76±10</td>
<td>75±8</td>
<td>78±7</td>
<td>81±9</td>
<td>0.06</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>66±13</td>
<td>72±10</td>
<td>68±13</td>
<td>80±13‡</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>74±16</td>
<td>69±15</td>
<td>64±13</td>
<td>76±15‡</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>62±8</td>
<td>65±5</td>
<td>67±6</td>
<td>65±8</td>
<td>0.14</td>
</tr>
<tr>
<td>Early peak filling rate, mL/ms</td>
<td>108±57</td>
<td>79±36*</td>
<td>63±26*</td>
<td>71±39*</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are mean±SD. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; LVMI, LV mass indexed to body surface area; and LVEF, LV ejection fraction.

*P<0.05 vs lean; †P<0.05 vs overweight and obese; ‡P<0.05 vs IGT.

for bivariate correlation analysis.
after adjustment for differences in serum triglycerides, body mass index, age, and gender (P<0.05 for trend).

Bivariate correlations revealed that myocardial triglyceride content was weakly associated with the homeostasis model assessment index (r=0.39, P<0.01), hepatic triglyceride content (r=0.30, P<0.01; Figure 3A), serum triglycerides, (r=0.19, P<0.05; Figure 3B), and visceral fat mass (r=0.35, P<0.01). Multiple linear regression analysis revealed that group and visceral fat were both independent determinants of myocardial triglyceride content (P<0.05), whereas age, BMI, and serum triglycerides were unrelated to myocardial triglyceride content once the effects of group and visceral fat were accounted for (Table 4).

Furthermore, the FFA AUC was significantly related to both myocardial (r=0.29, P<0.05) and hepatic (r=0.39, P<0.05) triglyceride content. Myocardial triglyceride content and FFA AUC were not related to LV ejection fraction or early diastolic filling dynamics.

Discussion

The major new findings from the present 1H-MRS study are 2-fold. First, in humans, IGT and T2D are accompanied by excessive myocardial triglyceride accumulation in vivo. Second, cardiac steatosis precedes the onset of diabetes mellitus and LV systolic dysfunction.

The present data confirm and extend the single previous study showing steatosis in the hearts of patients with diabetes mellitus.14 In that study, triglyceride staining was found to be common in the explanted hearts of diabetic patients undergoing cardiac transplantation for nonischemic cardiomyopathy. It was hypothesized that the combination of increased free fatty acid delivery to cardiomyocytes seen in obesity and T2D, coupled with the impaired fatty acid oxidation seen in heart failure, led to myocardial steatosis. Because all patients in that study had end-stage heart failure, it was unknown whether cardiac steatosis could precede the onset of heart failure. The noninvasive MRS technique allowed us to clearly demonstrate that this is indeed the case and that steatosis can even precede the onset of asymptomatic LV systolic dysfunction as determined by cardiac MRI. That cardiac steatosis was equivalent in subjects in the present study with IGT and those with frank T2D is consistent with an early mismatch between fatty acid delivery and mitochondrial fatty oxidation in the natural history of T2D.34

The present data set constitutes by far the largest published series to date of human cardiac 1H-MRS, which previously has been validated extensively in our laboratory.21,22 An important finding from the present study is that localized cardiac MRS provides additional information about human myocardial metabolism that cannot be gleaned from standard serum triglyceride measurements or even MRS measurement of hepatic triglyceride content. Although hepatic triglyceride content was elevated in patients with IGT and those with T2D, as in previous reports,30 hepatic triglyceride was not predictive of myocardial triglyceride. Thus, elevated levels of intracellular triglyceride in hepatocytes do not necessarily reflect elevated triglyceride levels in cardiac myocytes.

Table 3. MRI and MRS Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lean</th>
<th>Obese</th>
<th>IGT</th>
<th>T2D</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous fat, cm²</td>
<td>124±52</td>
<td>284±113*</td>
<td>282±123*</td>
<td>298±144*</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>Visceral fat, cm²</td>
<td>54±39</td>
<td>120±53*</td>
<td>132±36*</td>
<td>160±65*</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>Hepatic TG, F/W</td>
<td>1.1±0.9</td>
<td>4.3±5.2</td>
<td>8.3±11.1*</td>
<td>8.9±8.8*</td>
<td>&lt;0.01‡</td>
</tr>
<tr>
<td>Myocardial TG, F/W</td>
<td>0.46±0.30</td>
<td>0.81±0.46</td>
<td>0.95±0.60*</td>
<td>1.06±0.62*</td>
<td>&lt;0.01‡</td>
</tr>
</tbody>
</table>

Values are mean±SD. Hepatic TG indicates intrahepatic triglyceride; myocardial TG, intramyocardial triglycerides; and F/W, fat/water ratio.

*P<0.05 vs lean.
†ANOVA; ‡Kruskal-Wallis test.

Figure 2. Myocardial steatosis in IGT and T2D in humans. Myocardial triglyceride is higher in individuals with IGT and T2D (DM-2) vs lean individuals (‡P<0.01).

Figure 3. Common metabolic variables are poor predictors of myocardial triglyceride in humans. Neither hepatic triglyceride (r=0.3, P<0.01; A) nor serum triglycerides (r=0.55, P=0.22; B) provide adequate estimates of myocardial triglyceride in humans.
Potential Limitations
We considered whether the observed association between insulin resistance and cardiac steatosis might be confounded by age. Subjects with IGT and those with T2D were a decade older than the lean and obese subjects with normal glucose tolerance; however, age was not predictive of cardiac steatosis in multivariate models.

Patients with diabetes mellitus, recruited through Parkland Memorial Hospital, were treated with insulin for 2 weeks. Insulin is a well-known lipogenic agent, and it could artificially elevate myocardial triglyceride levels in the patients studied here. However, myocardial triglyceride levels among diabetic patients who were taking insulin before the examination and those who were newly diagnosed during the oral glucose tolerance test in the present study were uniformly elevated (data not shown).

Because the present study was cross-sectional, and all subjects had preserved LV systolic function, the present data do not establish a causal relationship between cardiac steatosis and cardiomyopathy in humans. Although the present MRI data provide evidence for diastolic dysfunction in the subjects with IGT and those with T2D, the dysfunction was dissociated from myocardial triglyceride content. Nevertheless, the present human data are entirely consistent with a large body of basic research that establishes a causal link between cardiac steatosis, the production of lipotoxic metabolites, and diabetic cardiomyopathy. Further prospective human studies of myocardial steatosis, cardiac structure, and function are warranted.

Clinical Implications
Because two thirds of Americans are overweight, with 73 million being glucose intolerant and 23 million having T2D, novel biomarkers for the early detection of subclinical cardiovascular disease are needed in these high-risk populations. 

1H-MRS is a sensitive new tool to detect cardiac steatosis, a preclinical abnormality that cannot be determined by standard diagnostic testing. Cardiac steatosis may identify a subset of individuals with IGT who are at high risk for subsequent development of nonischemic cardiomyopathy, and it constitutes a putative new therapeutic target to interrupt the early pathogenesis of this high-risk condition. In rodent models of cardiac steatosis, LV dysfunction can be rescued and the progression to dilated cardiomyopathy prevented by various pharmacological and genetic strategies that normalize myocardial triglyceride metabolism. Parallel translational human studies are necessary.

Conclusions
In humans, IGT is accompanied by cardiac steatosis, which precedes the onset of T2D and LV systolic dysfunction. Thus, lipid overstorage in human cardiac myocytes occurs early in the natural history of T2D and is evident in the absence of overt clinical heart failure.

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Disclosures
None.

References
The potential impact of this work relates to preclinical detection and prevention of obesity- and diabetes mellitus–related cardiomyopathy. Combined cardiac magnetic resonance imaging and spectroscopy provides a noninvasive diagnostic tool that allows precise measurement of myocardial lipid content and implicates lipid storage in the heart as an important component of impaired glucose tolerance even before the onset of frank diabetes or systolic dysfunction. Assessment of myocardial lipid content could lead to an enhanced understanding of the relationship between abnormalities in glucose handling and myocardial dysfunction, particularly in obese individuals, and potentially provide a new cardiac-specific measure for evaluating interventions that improve global glucose tolerance (eg, weight loss, exercise, and insulin sensitizers).
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