**Objectives**

This study was designed to evaluate myocardial substrate and high-energy phosphate (HEP) metabolism in asymptomatic men with well-controlled, uncomplicated type 2 diabetes with verified absence of cardiac ischemia, and age-matched control subjects, and to assess the association with myocardial function.

**Background**

Metabolic abnormalities, particularly an excessive exposure of the heart to circulating nonesterified fatty acids and myocardial insulin resistance are considered important contributors to diabetic cardiomyopathy in animal models of diabetes. The existence of myocardial metabolic derangements in uncomplicated human type 2 diabetes and their possible contribution to myocardial dysfunction still remain undetermined.

**Methods**

In 78 insulin-naive type 2 diabetes men (age 56.5 ± 5.6 years, body mass index 28.7 ± 3.5 kg/m², glycosylated hemoglobin A1c 7.1 ± 1.0%; expressed as mean ± SD) without cardiac ischemia and 24 normoglycemic control subjects (age 54.5 ± 7.1 years, body mass index 27.0 ± 2.5 kg/m², glycosylated hemoglobin A1c 5.3 ± 0.2%), we assessed myocardial left ventricular (LV) function by magnetic resonance imaging, and myocardial perfusion and substrate metabolism by positron emission tomography using H2 15O, carbon11C-palmitate, and 18-fluorodeoxyglucose 2-fluoro-2-deoxy-D-glucose. Cardiac HEP metabolism was assessed by phosphorous P 31 magnetic resonance spectroscopy.

**Results**

In patients, compared with control subjects, LV diastolic function (E/A ratio: 1.04 ± 0.25 vs. 1.26 ± 0.36, \( p = 0.003 \)) and myocardial glucose uptake (260 ± 128 nmol/ml/min vs. 348 ± 154 nmol/ml/min, \( p = 0.015 \)) were decreased, whereas myocardial nonesterified fatty acid uptake (88 ± 31 nmol/ml/min vs. 68 ± 18 nmol/ml/min, \( p = 0.021 \)) and oxidation (85 ± 30 nmol/ml/min vs. 63 ± 19 nmol/ml/min, \( p = 0.007 \)) were increased. There were no differences in myocardial HEP metabolism or perfusion. No association was found between LV diastolic function and cardiac substrate or HEP metabolism.

**Conclusions**

Patients versus control subjects showed impaired LV diastolic function and altered myocardial substrate metabolism, but unchanged HEP metabolism. We found no direct relation between cardiac diastolic function and parameters of myocardial metabolism. (J Am Coll Cardiol 2009;54:1524–32) © 2009 by the American College of Cardiology Foundation
Altered cardiac function, structure, and dimensions are common findings in asymptomatic patients with type 2 diabetes mellitus (T2DM), even in the absence of hypertension and coronary artery disease (CAD). These alterations are attributed to diabetic cardiomyopathy (DCM) (1). Diabetic cardiomyopathy is a multifactorial disease entity that is clinically characterized by an initial increase in left ventricular (LV) stiffness (2) and subclinical diastolic dysfunction. However, DCM may advance to compromised LV systolic function, with a high propensity to progress into overt congestive heart failure (3).

In animal models of diabetes, cardiac dysfunction coexists with increased myocardial nonesterified fatty acid (NEFA) utilization, triglyceride accumulation, and subsequent increased production of toxic intermediates, which, in the presence of hyperglycemia, contribute to increased formation of reactive oxygen species, mitochondrial uncoupling, decreased adenosine triphosphate (ATP) synthesis, mitochondrial dysfunction, and finally apoptosis (1,4). These deleterious processes are commonly referred to as gluco-lipotoxicity.

In patients with T2DM, it is technically challenging to demonstrate the existence of these abnormalities as causal mechanisms underlying DCM. Only a few studies have investigated myocardial glucose and NEFA metabolism in the human (pre-)diabetic heart, yielding conflicting results (1,5–14). Even fewer studies related altered cardiac substrate metabolism to function (8,9); however, these results are not unequivocal. Previously, we and others (15,16) showed decreased cardiac phosphocreatine (PCr)/ATP ratios in patients with T2DM, which was associated with diastolic dysfunction only in our study. Consequently, the inter-relation between myocardial substrate and energy metabolism and LV function is currently unclear in human T2DM.

The present study aimed to investigate the influence of T2DM on myocardial substrate and high-energy phosphate (HEP) metabolism and their relation to myocardial function using cardiac magnetic resonance (CMR), phosphorus-31 magnetic resonance spectroscopy ([31P]-MRS), and positron emission tomography (PET) in asymptomatic patients with recently diagnosed, uncomplicated T2DM. As CAD and ischemia influence myocardial substrate preference (9,17), only T2DM patients with verified absence of inducible ischemia as assessed by dobutamine stress echocardiography were included.

**Methods**

**Patients.** Patients and healthy control subjects were recruited by advertisements in local newspapers. Men with uncomplicated T2DM, age 45 to 65 years, were eligible. Inclusion criteria were a glycosylated hemoglobin A\(_1c\) (HbA\(_1c\)) level of 6.5% to 8.5% at screening, body mass index (BMI) of 25 to 32 kg/m\(^2\), and blood pressure not exceeding 150/85 mm Hg (with or without the use of antihypertensives). Patients were excluded when any history or complaints related to cardiovascular disease, diabetes, or any clinically noticeable disorder was present. Patients were also excluded if they used thiazolidinediones, fibrates, insulin, or other hormonal replacement therapy. Patients were screened at 2 separate occasions. A general screening was performed, consisting of a medical history, physical examination, echocardiogram, Ewing tests to exclude cardiac autonomic neuropathy, and fasting blood and urine analyses. When patients fulfilled the initial criteria, they underwent a dobutamine-stress echocardiography to exclude the presence of cardiac ischemia or arrhythmias at a separate visit. Following successful screening, participants entered a 10-week run-in period, during which their regular blood glucose lowering agents were transferred to glimepiride monotherapy and titrated until a stable dose was reached during 8 weeks prior to assessments to exclude possible confounding effects on myocardial metabolism of different agents. Mean HbA\(_1c\) levels at screening and at the end of the run-in period were comparable (data not shown).

Healthy men, age 45 to 65 years, with normal glucose metabolism, as assessed by a 75-g oral glucose tolerance test, were eligible as control subjects. Inclusion criteria were a BMI of 25 to 32 kg/m\(^2\) and blood pressure <150/85 mm Hg. Exclusion criteria were a history of or current cardiovascular disease, dyslipidemia, and the use of any prescribed medication. Control subjects underwent the same first screening visit as patients. CAD in control subjects was considered to be absent when there were no cardiac problems at rest and during exercise and when a normal electrocardiogram was present. The study was performed at 2 sites (Amsterdam and Leiden, the Netherlands). The protocol was approved by the Medical Ethics Committee of both centers and performed in full compliance with the Declaration of Helsinki.

**Biochemical analyses.** HbA\(_1c\) was determined by high-performance liquid chromatography (Menarini Diagnostics, Florence, Italy), with reference values of 4.3% to 6.1%.
N-terminal pro-B-type natriuretic peptide was measured using an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). The intraassay coefficient of variation was 1.5%, and the interassay coefficient of variation was 1.9%. The lower detection limit was 5 ng/l. The plasma concentration of malondialdehyde was determined in duplicate by high-performance liquid chromatography after alkaline hydrolysis and reaction with thiobarbituric acid (18). The intra-assay coefficient of variation was 5.7%. Ultra-sensitive C-reactive protein was determined by ELISA (DSL, Webster, Texas).

**Imaging.** The study protocol was performed on 2 different occasions, separated by 2 days, and consisted of CMR and [31P]-MRS on the first and PET on the second occasion or vice versa. All participants underwent CMR measurements. Due to the demanding protocol, [31P]-MRS was offered as an optional test. The PET measurements were performed in the first 60 patients entering the study. The patients did not take glucose lowering medication in the morning prior to assessments.

**CMR imaging protocol.** The CMR assessments were performed after an overnight fast at a single center (Leiden), using a 1.5-T whole-body MR scanner (Gyroscan ACS/NT15, Philips, Best, the Netherlands). During CMR examinations, blood pressure and heart rate were monitored and blood samples were collected to determine substrates. Rate pressure product was calculated as the product of systolic blood pressure and heart rate. The entire heart was imaged in the short-axis orientation using electrocardiographically gated breath-hold balanced steady-state free precession imaging (19).

Measures of systolic function were LV ejection fraction, cardiac work, and cardiac index (cardiac output/body surface area). The cardiac dimensions were LV end-diastolic volume index, LV end-systolic volume, LV stroke volume, and left ventricular mass (LVM) index (1). An electrocardiographically gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve for the determination of LV diastolic function parameters, including peak filling rates of the early filling phase (E) and the atrial contraction (A). The E/A ratio was calculated. Additionally, the peak deceleration of E (E-decel) was calculated (19). The LV filling pressures (E/e') were estimated (20). Images were analyzed quantitatively using dedicated software (MASS and FLOW, Medis, Leiden, the Netherlands).

**Phosphorus magnetic resonance spectroscopy.** A 100-mm-diameter surface coil was used to acquire electrographically triggered [31P]-MR spectra of the LV anterior wall with subjects in the supine position. Volumes of interest were selected by image-guided spectroscopy with 3-dimensional image selected in vivo spectroscopy. Shimming was performed automatically and tuning and matching of the 31P surface coil was performed manually. Technical details of data acquisition and spectral quantification were similar as described elsewhere (21). Briefly, spectroscopic volume size was typically 7 × 7 × 7 cm. Acquisitions were based on 192 averaged free-induction decays, and total acquisition time was 10 min. The [31P]-MR spectra were quantified automatically in the time domain using prior spectroscopic knowledge and were corrected for partial saturation effects and for the ATP contribution from blood in the cardiac chambers. The PCr/ATP ratios of the spectra were calculated and used as a parameter representing myocardial HEP metabolism (22).

**PET imaging protocol.** The PET examinations were performed after an overnight fast at a single center (Amsterdam) using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, Tennessee). Patients received 2 venous catheters: 1 in an antecubital vein and 1 in the vein of the opposite hand, with the latter being wrapped into a heated blanket to obtain arterialized blood. During procedures, patients were monitored by telemetry, and blood pressure was measured at 5-min intervals. Positron emission tomography was used to measure myocardial blood flow with H215O, myocardial NEFA uptake (MFAU), beta-oxidation (MFAO), and esterification (MFAE) with carbon 11C-palmitate, and myocardial metabolic rate of glucose uptake (MMRglu) with 18-fluorodeoxyglucose-2-fluoro-2-deoxy-D-glucose (18FDG). Perfusion and NEFA metabolism were assessed in the fasting state, whereas MMRglu was measured during euglycemic-hyperinsulinemic clamp procedure. Following a 10-min transmission scan for attenuation correction, H215O was injected (t = 10 min), and a 10-min dynamic emission scan (40 frames with increasing frame length) was acquired. Subsequently, a 30-min dynamic emission scan (34 frames) was performed following 11C-palmitate injection (t = 35 min). Hereafter, the clamp was started (t = 65 min), as described elsewhere (23), to approximate an isometabolic steady state (plasma glucose level 5 mmol/l) and measure whole-body insulin sensitivity. At steady state (around t = 155 min), following a new transmission scan, 18FDG was injected, and a 60-min dynamic emission scan (40 frames) was acquired. Blood samples were collected during 11C-palmitate and 18FDG scans at pre-defined time points to measure glucose, NEFA, lactate, lipids, and insulin levels. In addition, 11CO2 was measured during the 11C-palmitate scan (14). Total radiation exposure was 4.87 mSv.

**PET image analysis.** The PET data were quantitatively reconstructed using filtered backprojection applying all appropriate corrections. To generate myocardial time-activity curves, regions of interest were defined on resliced LV short-axis (summed) 11C-palmitate and 18FDG images and subsequently projected onto the dynamic images. The regions of interest were drawn as previously described (24) and grouped for further analysis. Myocardial segments exposed to liver spill-in were omitted from the analysis of 11C-palmitate data. Additional regions of interest were defined in left and right ventricular chambers for 11C-palmitate and H215O image-derived input functions. A separate aorta ascendance region of interest was defined for...
the $^{18}$FDG image-derived input function. Myocardial blood flow was determined using the standard single-tissue compartment model (25). The $^{11}$C-palmitate time-activity curves were analyzed using a 3-tissue plasma input kinetic model, which, together with plasma NEFA concentrations, enabled calculation of MFAU, MFAO, and MFAE (26). The $^{11}$C-palmitate image-derived input function was corrected for $^{11}$CO$_2$ metabolites and difference between plasma and whole blood concentrations as described elsewhere (14). This model is similar to that described by Bergmann et al. (27), but with a reduced number of free parameters, thereby increasing precision of derived estimates (Online Fig. 1). Then, MMRglu was calculated by multiplying the net influx constant for $^{18}$FDG, $k_i$, by the mean plasma glucose concentration. For determination of $k_i$, Patlak graphical analysis was used (28).

Statistical analysis. Values are expressed as mean ± SD or median (interquartile range). Non-normally distributed data were logarithmically transformed. Comparisons between patients and control subjects were made using the independent-sample $t$ test. Linear regression was used to adjust for BMI differences between groups. Univariate and multiple analyses with a forward selection procedure were performed. The goal of these analyses was to determine which factors were responsible for the difference in LV diastolic function (E/A and E-decpeak) between groups. We employed a 2-step strategy for the selection of variables. The first step was that a variable had to be significantly different between groups. If so, in univariate analysis there had to be an association between this variable and the dependent variable with $p < 0.1$. The variables fulfilling these criteria were then entered in a forward multivariable regression analysis, and those with $p < 0.05$ were considered independently related to the dependent variable. Analyses were performed with SPSS software version 15.0 (SPSS Inc., Chicago, Illinois). A 2-tailed probability value $<0.05$ was considered significant.

Results

Subject characteristics, hemodynamics, and LV dimensions and function. A total of 173 patients underwent the general screening; 96 patients fulfilled all inclusion criteria. Therefore, 96 patients underwent dobutamine-stress echocardiography. Sixteen patients were excluded (17.7%) as result of a positive test, and 2 patients withdrew before measurements, leaving a total of 78 patients entered into the study.

Patients and control subjects were similar with respect to age, but patients had a slightly higher BMI and waist circumference (Table 1). The lipids profiles were different between patients and control subjects (Table 1). Table 2 shows hemodynamic and cardiac parameters. Blood pressures and heart rates were within the normal range, but slightly higher in patients than in control subjects. Patients had a significantly lower LV end-diastolic volume (index) and LV stroke volume than control subjects in the presence of comparable LVM index, LV ejection fraction, cardiac index, and cardiac work. Patients had significantly decreased diastolic functional parameters. Mean E/Ea and N-terminal pro–B-type natriuretic peptide, both estimates of LV filling pressure, were not different between both groups. In T2DM patients, the relation between LV end-diastolic volume (index) and E/Ea was shifted toward decreased compliance (Fig. 1).

Myocardial substrate and HEP metabolism. All $^{18}$FDG and all but 1 of the $^{15}$O scans were appropriate for analysis. Due to technical difficulties, $^{11}$C-palmitate images from 8 patients and 4 control subjects were not available for analysis. Due to the demanding protocol or insufficient spectral quality (Cramér–Rao standard deviation $>20\%$) (21), $^{31}$P-MRS data were available for analysis in 35 patients and 19 control subjects.

Table 3 lists the fasting biochemical variables obtained before the onset of $^{15}$O and $^{11}$C-palmitate PET and during euglycemic hyperinsulinemia (i.e., during $^{18}$FDG-PET). Myocardial blood flow was similar between patients and control subjects (0.884 ± 0.213 g/ml/min vs. 0.894 ± 0.215 g/ml/min, $p = 0.804$). In patients, MFAU (88 ± 31 nmol/ml/min vs. 68 ± 18 nmol/ml/min, $p = 0.021$) and MFAO (85 ± 30 nmol/ml/min vs. 63 ± 19 nmol/ml/min, $p = 0.007$) were higher than in control subjects, whereas the negligible MFAE was lower in patients versus control subjects (2.3 ± 4.5 nmol/ml/min vs. 5.3 ± 5.4 nmol/ml/min, $p = 0.028$) (Fig. 2A). Myocardial metabolic rate of glucose intake was lower in patients than in control subjects.

### Table 1 Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n = 24)</th>
<th>Type 2 Diabetic Patients (n = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>54.5 ± 7.1</td>
<td>56.8 ± 6.6</td>
</tr>
<tr>
<td>Time since diagnosis of diabetes, yrs</td>
<td>NA (4–6)</td>
<td></td>
</tr>
<tr>
<td>Current smoker, n/N</td>
<td>0/24</td>
<td>17/78*</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>27.0 ± 2.5</td>
<td>28.7 ± 3.5†</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>99 ± 9</td>
<td>104 ± 10†</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.3 ± 0.2</td>
<td>7.1 ± 1.0*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>5.3 ± 0.7</td>
<td>4.7 ± 1.0†</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>3.4 ± 0.65</td>
<td>2.7 ± 0.7*</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.4 (1.3–1.6)</td>
<td>1.1 (0.9–1.3)*</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>0.9 (0.7–1.1)</td>
<td>1.5 (1.0–2.2)*</td>
</tr>
</tbody>
</table>

Data are mean ± SD or median (interquartile range). $^*$p < 0.001, †p < 0.01, ‡p < 0.05.

ACE = angiotensin-converting enzyme; HbA1c = glycosylated hemoglobin A1c; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NA = not applicable.
(260 ± 128 nmol/ml/min vs. 348 ± 154 nmol/ml/min, p = 0.015) (Fig. 2B). Following correction for BMI differences between groups, only the difference in MFAE was no longer statistically significant. Analysis of T2DM patients and control subjects in whom the PCr/ATP ratio was available did not reach significance: MFAU (76 ± 24 nmol/ml/min vs. 68 ± 19 nmol/ml/min, p = 0.303), MFAO (74 ± 23 nmol/ml/min vs. 63 ± 19 nmol/ml/min, p = 0.127), and MMRglu (284 ± 149 nmol/ml/min vs. 363 ± 161 mol/ml/min, p = 0.116). Moreover, the PCr/ATP ratio was comparable in the 2 groups (2.09 ± 0.33 vs. 2.12 ± 0.32, p = 0.817) (Fig. 2C).

**Correlations and determinants of LV diastolic function.** Whole-body insulin sensitivity and cardiac substrate or HEP metabolism were not directly associated with LV function. In pooled analysis, the value for the whole body insulin sensitivity adjusted during the steady state was positively correlated with MMRglu (r = 0.452, p = 0.001), whereas an inverse correlation was found with NEFA (r = −0.361, p = 0.001), MFAU (r = −0.255, p = 0.042), and MFAO (r = −0.288, p = 0.021). We found NEFA was negatively associated with MMRglu (r = −0.562, p < 0.001). Forward multiple regression analysis revealed heart rate, HbA1c, and diastolic blood pressure as independent determinants of E/A ratio (R² = 0.29, p < 0.001) and E-decpeak (R² = 0.27, p < 0.001) (Table 4).

**Discussion**

This study is novel in several ways. To our knowledge, this is: 1) the largest group of well-characterized male T2DM patients with 2) verified absence of (inducible) cardiac ischemia who had extensive evaluation of 3) combined cardiac substrate and HEP metabolism as well as function with CMR and PET technology under standardized conditions. In addition, 4) this study is the first to show convincingly an increase in myocardial fatty acid metabolism in T2DM patients, whereas 5) no direct relation was found between cardiac diastolic function and parameters of myocardial substrate or energy metabolism.

**Myocardial function.** The findings of the present study are in line with previous reports, showing LV diastolic dysfunction in asymptomatic patients with T2DM (15). The strength of the present study is the a priori exclusion of participants with inducible ischemia, as ischemia is a critical determinant of both cardiac function and substrate and energy metabolism (9,17). Previous studies evaluating LV function and dimensions in human diabetes have mainly used echocardiography, which is hampered by several well-known limitations, particularly in overweight/obese populations (29). We used CMR, which represents the gold standard for reproducible, observer-independent quantitative assessment of LV volume and mass and has been shown to be an important tool for the assessment of diastolic and systolic function together with excellent accuracy and reproducibility (29). The observed LV diastolic dysfunction and decreased LV volumes, in the presence of normal LVM, systolic function, and cardiac work, unveil early abnormalities in LV function and compliance in the course of DCM. Thus, the functional changes cannot be explained by in-
increased LVM, even after adjustment for BMI, or by raised LV pressure because the estimates of LV filling pressure measured were similar in both groups.

**Myocardial substrate metabolism.** Myocardial substrate metabolism was previously studied in humans with various degrees of glucometabolic abnormalities and comorbidities (5–14). These small-sized studies used PET or single-photon emission computed tomography to assess glucose and NEFA metabolism, applying several tracers with different isotopes and metabolic fates, but they also used various approaches to data analysis. Due to these differences in populations and methodology, different, often conflicting results were obtained (5–8,14). In the present study, T2DM patients had increased NEFA uptake and oxidation, which is compatible with the data derived from animal models of DCM (1,30). Increased myocyte NEFA metabolism can be caused by elevated plasma NEFA supply due to unsuppressed lipolysis from insulin-resistant adipocytes (31,32). Interestingly, similar to findings by others in comparable populations (9–11), but in contrast to most animal studies (30), fasting plasma NEFA levels in our T2DM patients were similar to control subjects. In addition, myocardial perfusion was comparable between both study groups; thus, these factors do not explain the observed increases in cardiac NEFA metabolism. Increased NEFA metabolism can, however, be explained by alterations at a cellular level. In rats fed a high-fat diet, the fatty acid transporter CD36 was relocated to the sarcolemma, and basal phosphorylation of a mediator of CD36, that is, protein kinase B, was increased (33). In addition, in Zucker diabetic fatty rats, increased myocardial NEFA use was found, and this was shown to be related to increased peroxisome proliferator-activated receptor alpha and/or its coactivator PGC-1 activation (34,35). Finally, in ZDF rats, we found increased messenger ribonucleic acid levels of the mitochondrial NEFA shuttling enzyme carnitine palmitoyltransferase, which subsequently may lead to enhanced NEFA oxidation (33). Furthermore, NEFA levels under hyperinsulinemia were significantly higher in patients than in control subjects and were inversely associated with whole-body insulin sensitivity and the myocardial metabolic rate of glucose uptake, confirming previous findings (9,12,13). Theoretically, increased cardiac NEFA metabolism should be paralleled by a decrease in myocardial glucose uptake, measured as insulin-stimulated 18FDG uptake. However, studies in T2DM subjects without CAD have reported both decreased (9,11–13) and

### Table 3  Biochemical and Metabolic Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>Type 2 Diabetic Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting</strong></td>
<td>n = 24</td>
<td>n = 78</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>5.3 (5.0–5.6)</td>
<td>8.3 (7.1–10.0)*</td>
</tr>
<tr>
<td>Plasma nonesterified fatty acids, mmol/l</td>
<td>0.46 (0.37–0.52)</td>
<td>0.50 (0.40–0.62)</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>28 (19–33)</td>
<td>64 (36–92)*</td>
</tr>
<tr>
<td>Plasma lactate, mmol/l</td>
<td>0.8 (0.7–0.9)</td>
<td>1.2 (1.0–1.5)*</td>
</tr>
<tr>
<td>NT-proBNP, ng/l</td>
<td>28 (21–76)</td>
<td>27 (20–42)</td>
</tr>
<tr>
<td>usCRP, mg/l</td>
<td>3.0 (1.7–6.4)</td>
<td>3.8 (2.4–6.1)</td>
</tr>
<tr>
<td>Malondialdehyde, μmol/l</td>
<td>6.1 ± 0.7</td>
<td>9.8 ± 2.3*</td>
</tr>
<tr>
<td><strong>During hyperinsulinemia</strong></td>
<td>n = 19</td>
<td>n = 72</td>
</tr>
<tr>
<td>Plasma nonesterified fatty acids, mmol/l</td>
<td>0.04 (0.02–0.05)</td>
<td>0.08 (0.05–0.16)*</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>513 ± 64</td>
<td>583 ± 143</td>
</tr>
<tr>
<td>Plasma lactate, mmol/l</td>
<td>1.1 (0.9–1.3)</td>
<td>1.1 (1.0–1.3)</td>
</tr>
<tr>
<td>M/I value, (mg/kg · min)/(pmol/l)</td>
<td>0.99 (0.72–1.65)</td>
<td>0.46 (0.24–0.74) *</td>
</tr>
</tbody>
</table>

Data are mean ± SD or median (interquartile range). *p < 0.001. M/I value = whole body insulin sensitivity adjusted during the steady state; NT-proBNP = N-terminal pro–B-type natriuretic peptide; usCRP = ultrasensitive C-reactive protein.

**Figure 2** Myocardial Substrate and High-Energy Phosphate Metabolism

Myocardial fatty acid uptake (MFAU), myocardial fatty acid oxidation (MFAO), and myocardial fatty acid esterification (MFAE) (A): the metabolic rate of glucose uptake (MMRglu) (B); and phosphocreatine/adenosine triphosphate (PCr/ATP) ratio (C) in control subjects (open bars) and T2DM patients (solid bars). *p < 0.001, †p < 0.01, ‡p < 0.05. Abbreviation as in Figure 1.
unaltered (10) cardiac glucose uptake. Again, differences in population characteristics and methodological issues preclude comparison of these studies. We found decreased glucose uptake, which is compatible with NEFA substrate competition, decreased myocardial insulin signaling (32), and/or reduced membrane-bound GLUT4 (35). Altogether, these findings implicate that the nonischemic myocardium in uncomplicated T2DM is resistant to insulin-mediated glucose uptake and that cardiac NEFA metabolism is augmented.

Myocardial substrate and HEP metabolism in relation to function. Studies aimed at linking cardiac substrate and HEP metabolism and function in humans with glucometabolic disorders are largely lacking. Using iodine (I) 123 heptadecanoic acid single-photon emission computed tomography, Turpeinen et al. (8) reported decreased myocardial NEFA uptake and kinetics in subjects with impaired glucose tolerance and T2DM, relative to type 1 diabetes mellitus patients and control subjects, which was associated with LVM measured by ultrasound. There were no functional differences between the groups, and no correlation was reported between functional measurements and [123I]-heptadecanoic acid metabolism. Iozzo et al. (9) reported a weak correlation ($r = 0.38$, $p < 0.02$) between insulin-stimulated myocardial glucose uptake and ejection fraction in a pooled analysis of control subjects, patients with type 1 diabetes mellitus, CAD patients without T2DM, and patients with T2DM without CAD. These investigators suggested that the impairment of myocardial glucose uptake as such might compromise myocardial function. In their combined group of subjects of various age groups, the ejection fraction varied substantially, with approximately 50% of participants having an ejection fraction $<40\%$ with extreme values as low as 10% (9). In our study, the ejection fraction in T2DM subjects and control subjects was similar, with small standard deviations in both groups, which may have precluded the detection of an association of LV function and myocardial glucose uptake. The inter-relation of altered myocardial substrate handling, HEP metabolism, and LV function is complex and depends on many factors, including additional comorbidities, such as hypertension and cardiac autonomic neuropathy, and the presence or absence of stress or ischemia.

Previously, we and others (15,16) reported decreased myocardial PCr/ATP ratio in T2DM patients relative to control subjects. In the present study, we could not confirm these previous findings. Several differences in patient characteristics between the present and the 2 other studies, such as age range (36), BMI differences (37), inclusion of only men or both sexes (38), glycemic control, and fasting NEFA levels, may have led to these discrepant findings. Because altered myocardial substrate metabolism may affect cardiac PCr/ATP (39), we argue that the insufficient contrast between the present subgroups regarding cardiac substrate metabolism may have potentially contributed to the finding of comparable myocardial PCr/ATP values in both groups.

In our previous study (15), a relation was found between myocardial LV diastolic function and cardiac PCr/ATP. This relation was not found by Scheuermann-Freestone et al. (16), who found a similar LV diastolic function in T2DM patients and control subjects. Likewise, we did not find a relation between LV diastolic function and cardiac PCr/ATP in the present study. Again, the lack of contrasts between T2DM patients and control subjects, with respect to LV diastolic function (16) or cardiac PCr/ATP (in the present study), may have precluded the finding of the previously observed association. Interestingly, a recent study in Zucker diabetic fatty rats showed that not myocardial energy metabolism per se, but rather cardiac remodeling affected the observed cardiac dysfunction (40).

Although we did not find a direct association between altered myocardial substrate metabolism and LV functional changes in T2DM, an indirect effect of cardiac metabolic derangements may influence LV function. Accordingly, recent data indicate that diabetes-related metabolic changes might indirectly impair LV diastolic function by inducing tissue oxidative stress, thereby promoting apoptosis and oxidative modification of contractile proteins, and/or by stimulating cardiac macrophage infiltration leading to local inflammation and fibrosis (41).
Study limitations. Due to the intrindividual diurnal fluctuations of substrate plasma levels in T2DM, real-life cardiac substrate metabolism may be difficult to establish. The euglycemic–hyperinsulinemic clamp guarantees an iso-metabolic state in experimental groups, allowing comparisons of cardiac glucose metabolism between groups. Inasmuch as hyperinsulinemia will lead to direct uptake of plasma NEFA into nonadipose tissues other than the myocardium, NEFA metabolism was assessed in the fasting state and therefore does not allow the establishment of a direct association of cardiac NEFA and glucose metabolism. Furthermore, the present study does not address myocardial efficiency because the used tracers do not measure myocardial oxygen consumption. Further studies are needed to settle this issue in T2DM patients. Finally, the assessment of only men limits generalization of the findings.

Conclusions

In the absence of myocardial ischemia, patients with uncomplicated T2DM showed impaired LV diastolic function and decreased compliance, in addition to whole-body and myocardial insulin resistance, collectively leading to altered myocardial substrate metabolism, favoring NEFA over glucose as a substrate. We did not find a direct relation between altered LV diastolic function and myocardial substrate or HEP metabolism in this early stage of nonischemic DCM. Large-scale longitudinal studies using similar combined measurements should establish the influence of early metabolic and functional changes on the development of clinically relevant cardiac disease in T2DM over time, including congestive heart failure and cardiac ischemia, and further define the efficacy of targeted interventions to halt the progression of compensated DCM into overt cardiac disease.

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REFERENCES

Key Words: diabetes mellitus □ cardiomyopathy □ metabolism □ magnetic resonance imaging □ tomography □ positron emission.

APPENDIX

For supplementary discussions of the 11C-palmitate kinetic analysis, including Online Figure 1 and [31P]-MRS technique, please see the online version of this article.