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Myocardial Steatosis Is an Independent Predictor of Diastolic Dysfunction in Type 2 Diabetes Mellitus

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Objectives The purpose of this study was to compare myocardial triglyceride content and function between patients with uncomplicated type 2 diabetes mellitus (T2DM) and healthy subjects within the same range of age and body mass index (BMI), and to study the associations between myocardial triglyceride content and function.

Background T2DM is a major risk factor for cardiovascular disease. Increasing evidence is emerging that lipid oversupply to cardiomyocytes plays a role in the development of diabetic cardiomyopathy, by causing lipotoxic injury and myocardial steatosis.

Methods Myocardial triglyceride content and myocardial function were measured in 38 T2DM patients and 28 healthy volunteers in the same range of age and BMI by proton magnetic resonance (MR) spectroscopy and MR imaging, respectively. Myocardial triglyceride content was calculated as a percentage relative to the signal of myocardial water.

Results Myocardial triglyceride content was significantly higher in T2DM patients compared with healthy volunteers ($0.96 \pm 0.07\%$ vs. $0.65 \pm 0.05\%$, $p < 0.05$). Systolic function did not significantly differ between both groups. Indexes of diastolic function, including the ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate (E/A) and E peak deceleration, were significantly impaired in T2DM compared with those in healthy subjects (1.08 ± 0.04 ml/s^2 $\times 10^{-3}$ vs. 1.24 ± 0.06 ml/s^2 $\times 10^{-3}$ and 3.6 ± 0.2 ml/s^2 $\times 10^{-3}$ vs. 4.4 ± 0.3 ml/s^2 $\times 10^{-3}$, respectively, $p < 0.05$). Multivariable analysis indicated that myocardial triglyceride content was associated with E/A and E peak deceleration, independently of diabetic state, age, BMI, heart rate, visceral fat, and diastolic blood pressure.

Conclusions Myocardial triglyceride content is increased in uncomplicated T2DM and is associated with impaired left ventricular diastolic function, independently of age, BMI, heart rate, visceral fat, and diastolic blood pressure. (J Am Coll Cardiol 2008;52:1793–9) © 2008 by the American College of Cardiology Foundation

Type 2 diabetes mellitus (T2DM) is a major risk factor for cardiovascular disease and early death (1,2). The pathophysiology of nonischemic diabetic cardiomyopathy is complex, and the exact mechanisms of disease remain partly unknown (2). Increasing evidence is emerging that lipid oversupply to cardiomyocytes, which may lead to lipotoxic injury, plays a role in the development of diabetic cardiomyopathy (3–5). Increased fatty acid (FA) fluxes arising from the disproportionate amount of insulin resistant (visceral) adipose tissue lead to excessive FA delivery and uptake in the heart. This FA uptake exceeds the oxidizing requirements of the organ, giving rise to fatty acyl-CoA esters, diacylglycerol, and ceramide as intermediates (5). Increasing evidence exists that accumulation of these FA intermediates causes mitochondrial dysfunction and reactive oxygen species, giving rise either directly to cell damage and apoptosis or indirectly through the induction of inflammatory cascades, which leads to myocardial dysfunction (6–9). In animal models, antisteatotic treatment with thiazolidinediones reduced myocardial triglyceride accumulation and ceramide content, and prevented myocardial dysfunction (7). Recently, it has been demonstrated in a heterogeneous group of T2DM patients that myocardial triglyceride content was increased compared with that in young, lean, healthy control subjects

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Abbreviations and Acronyms

A = atrial contraction
BMI = body mass index
E = early diastolic filling phase
E/A = ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate
E/Ea = estimation of left ventricular filling pressures
LV = left ventricle/ventricular
MR = magnetic resonance
MRI = magnetic resonance imaging
(NE)FA = (nonesterified) fatty acids
T2DM = type 2 diabetes mellitus

(10). However, direct correlations between myocardial triglyceride accumulation and heart function could not be established. To investigate the net contribution of T2DM to myocardial triglyceride accumulation and the associated functional consequences, it is essential to select controls within the same range of age and body mass index (BMI). In addition, to investigate the association between myocardial triglyceride accumulation and heart function, underlying ischemic heart disease should be excluded.

Left ventricular (LV) diastolic function can be assessed using flow velocity encoded magnetic resonance imaging (MRI). Using this technique, myocardial diastolic functional parameters have been shown to associate with myocardial triglyceride accumulation in healthy volunteers (11). Furthermore, in explanted hearts of obese and T2DM patients with end-stage heart failure, lipid staining was a common finding (4).

In addition to myocardial accumulation of triglycerides, T2DM is associated with increased visceral adipose tissue, which associates with elevations of circulating plasma triglycerides and nonesterified fatty acid (NEFA) levels, and thus contributes to lipid overexposure to nonadipose tissue compartments.

Therefore, the purpose of the present study was to compare myocardial and hepatic triglyceride content and myocardial function between patients with uncomplicated T2DM and healthy subjects within the same range of age and BMI and to study the associations between myocardial triglyceride accumulation and heart function.

Methods

Subjects. Forty-one male T2DM patients were included in this study, which was approved by the local ethics committee. Hormonal status or use of contraceptives may affect lipid metabolism in women. Plasma estrogen levels influence lipid metabolism (including plasma lipid levels, adipose tissue), and gender differences in expression of certain cell surface receptors/transporters of fatty acids have been reported (12,13). Therefore, we decided to exclude women to avoid possible confounding influences of fluctuation in lipid metabolism in women on hepatic and myocardial triglyceride accumulation. All participants signed informed consent. Patients were recruited by advertisements in local newspapers according to the following inclusion criteria: 1) age 45 to 65 years; 2) T2DM diagnosed according to World Health Organization criteria (14) and treated with sulfonylurea derivates in stable doses; 3) glycated hemoglobin below 8.5%; and 4) sitting blood pressure <150/85 mm Hg, with or without antihypertensive drugs. Exclusion criteria included impaired hepatic function or a history of liver disease; substance abuse; known cardiovascular disease or diabetes-related complications, including proliferative retinopathy; autonomic neuropathy, as excluded by Ewing's tests (15); microalbuminuria, as excluded by measurements of albumin/creatinine ratio in a urine sample; and all contraindications for MRI. Furthermore, to avoid interference with the main study parameters, patients on lipid lowering therapy (such as statins, fibrates) were excluded. Most importantly, however, myocardial ischemia was excluded by means of high-dose dobutamine stress echocardiography.

Thirty healthy male control subjects within the same range of age (45 to 65 years) and BMI (25 to 32 kg/m^2) as the patient group were recruited by advertisements in the local newspapers. Subjects were included when they fulfilled the following criteria: no known acute or chronic disease based on history and physical examination and standard laboratory tests (blood counts, fasting blood glucose, lipids, serum creatinine, liver enzymes, and electrocardiogram). Exclusion criteria included chronic use of any drug, substance abuse, hypertension, and impaired glucose tolerance (as excluded by a 75-g oral glucose tolerance test) (16).

Magnetic resonance (MR) spectroscopy. All subjects underwent MR scanning in the morning for assessment of ectopic triglyceride content and heart function after an overnight fast and after blood sampling. All MR studies were performed with the use of a 1.5-T whole-body MR scanner (Gyroscan ACS/NT15, Philips, Best, the Netherlands) with subjects in the supine position at rest.

Myocardial ^{1}H-MR spectra were obtained as described previously (17). Briefly, the body coil was used for radiofrequency transmission and a 17-cm diameter circular surface coil was used for signal reception. An 8-ml voxel was positioned in the interventricular septum. Spectroscopic data acquisition was double triggered using electrocardiographic triggering and respiratory navigator echoes to minimize breathing influences (17). Water suppressed spectra were acquired for detection of the triglyceride signals at end systole, with an echo time of 26 ms and a repetition time of at least 3,000 ms, and 1,024 data points were collected using a 1,000-Hz spectral width and averaged over 128 acquisitions. Without changing any parameter, spectra without water suppression with a repetition time of 10 s and 4 averages were obtained from the same voxel, to be used as an internal standard.

^{1}H-MR spectroscopy of the liver was performed with an 8-ml voxel positioned in the liver, avoiding gross vascular structures and adipose tissue depots. Spectra were obtained using the same parameters as described for myocardial ^{1}H-MR spectroscopy. Only 64 averages were collected with water suppression. All ^{1}H-MR spectroscopic data were

fitted using Java-based MR user interface software (jMRUI version 2.2, developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium) (18) as described previously (17). Myocardial and hepatic triglyceride content was calculated as a percentage relative to water as (signal amplitude of triglyceride)/(signal amplitude of water) × 100.

MR imaging. All images were analyzed quantitatively using dedicated software (FLOW or MASS, Medis, Leiden, the Netherlands). The entire heart was imaged in short-axis orientation using electrocardiographically gated breath-holds with a sensitivity encoding balanced turbo-field echo sequence. Imaging parameters included the following: echo time = 1.7 ms, repetition time = 3.4 ms, flip-angle = 35°, slice thickness = 10 mm with a gap of 0 mm, field of view = 400 × 400 mm, reconstructed matrix size = 256 × 256. LV ejection fraction was assessed for the determination of LV systolic function. In addition, LV mass/(end-diastolic) volume ratio was calculated

An electrocardiographically gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve for determination of LV diastolic function. Imaging parameters included the following: echo time = 5 ms, repetition time = 14 ms, flip-angle = 20°, slice thickness = 8 mm, field of view = 350 × 350 mm, matrix size = 256 × 256, velocity encoding gradient = 100 cm/s, scan percentage = 80%. The resulting biphasic diastolic inflow pattern consists of 2 peaks, representing the early filling phase and the atrial contraction. Analysis of the early filling phase and the atrial contraction was performed by calculating their peak filling rates and ratio of the peak filling rates (E/A). Furthermore, the peak deceleration gradient of the early filling phase (E deceleration peak) was calculated automatically. In addition, an estimation of LV filling pressures (E/Ea) was assessed as described earlier (19). During MRI, blood pressure and heart rate were measured.

Abdominal visceral fat depots were quantified by MRI (20). A turbo spin echo imaging protocol was used and imaging parameters included the following: echo time = 11 ms, repetition time = 168 ms, flip angle = 90°, slice thickness = 10 mm. Three consecutive transverse images were obtained during 1 breath hold, with the middle image at a level just above the fifth lumbar vertebra. The volumes of the visceral fat depots of all slices were calculated by converting the number of pixels to square centimeters multiplied by the slice thickness. The total volume of the fat depots was calculated by summing the volumes of all three slices.

Assays. All samples were analyzed at 1 certified central laboratory (Amsterdam, the Netherlands). Plasma glucose was measured using a hexokinase-based technique (Roche Diagnostics, Mannheim, Germany); glycated hemoglobin was determined by high-performance liquid chromatography (Menarini Diagnostics, Florence, Italy; reference values: 4.3% to 6.1%). Plasma total cholesterol, high-density lipoprotein cholesterol, and triglycerides were determined using enzymatic colorimetric methods (Modular, Hitachi, Japan). Low-density lipoprotein cholesterol was calculated using Friedewald's formula. Insulin was measured by an immunoradiometric assay (Bayer Diagnostics, Mijdrecht, the Netherlands). NEFAs were assessed using enzyme-linked immunoabsorbent assay (WAKO Chemicals, Neuss, Germany).

Statistical analysis. Statistical analysis was performed using SPSS for Windows version 12.0 (SPSS Inc., Chicago, Illinois). Data are expressed as mean ± standard error when normally distributed; data not normally distributed are expressed as median (interquartile range). Non-normally distributed data were log-transformed, and unpaired *t* tests (or, when appropriate, nonparametric tests) were used for comparisons. To detect determinants of myocardial triglyceride content and LV function, univariate and multivariable linear regression analyses were performed. Values of $p < 0.05$ were considered statistically significant.

Results

Baseline characteristics of patients and healthy subjects are displayed in Table 1. The MR scan protocol could not successfully be completed due to technical constraints in 3 patients and in 2 healthy subjects. Therefore, data obtained from 38 patients and 28 healthy subjects were used for analysis. Mean systolic blood pressure in both groups was well within the normal range, although it was higher in patients than in controls (127 ± 2 mm Hg vs. 116 ± 2 mm Hg, $p < 0.05$). Although autonomic neuropathy was absent, documented by Ewing's tests in the patients, their resting heart rate was increased relative to that in controls (median [interquartile range]: 65 [62 to 72] beats/min vs. 59 [52 to 63] beats/min, $p < 0.05$).

Myocardial and hepatic triglyceride content. Myocardial triglyceride content was significantly higher in patients with T2DM compared with healthy volunteers (0.96 ± 0.07% vs.

Table 1 Clinical and Biochemical Characteristics

	Healthy Subjects (n = 28)	Patients With T2DM (n = 38)
Age (yrs)	54 ± 1	57 ± 1
Body mass index (kg/m^2)	26.9 ± 0.5	28.1 ± 0.6
Systolic blood pressure (mm Hg)	116 ± 2	127 ± 2*
Diastolic blood pressure (mm Hg)	73 ± 2	76 ± 1
Heart rate (beats/min)	59 (52–63)	65 (62–72)*
Plasma glucose (mmol/l)	5.3 ± 0.1	9.1 ± 0.3*
Plasma insulin (pmol/l)	35 (24–51)	55 (35–100)*
Glycated hemoglobin (%)	5.33 ± 0.05	7.21 ± 0.17*
Plasma triglycerides (mmol/l)	1.0 (0.8–1.3)	1.6 (1.1–2.8)*
Nonesterified fatty acids (mmol/l)	0.45 ± 0.04	0.49 ± 0.03
High-density lipoprotein cholesterol (mmol/l)	1.39 ± 0.06	1.09 ± 0.04*
Low-density lipoprotein cholesterol (mmol/l)	3.3 (3.0–3.7)	2.9 (2.6–3.4)*
Total cholesterol (mmol/l)	5.29 ± 0.14	5.00 ± 0.13

Data are mean ± standard error or median (interquartile range). *$p < 0.05$ compared with healthy subjects.
T2DM = type 2 diabetes mellitus.

Table 2 Magnetic Resonance Study Parameters

	Healthy Subjects	Patients With T2DM
Myocardial triglyceride content (%)	0.65 ± 0.05	0.96 ± 0.07*
Hepatic triglyceride content (%)	2.2 (1.2–3.8)	38.6 (2.7–24.3)*
Visceral fat (ml)	284 ± 24	420 ± 31*
LV mass (g)	108 ± 5	105 ± 3
LV mass/volume ratio (g/ml)	0.09 ± 0.02	0.11 ± 0.02*
LV ejection fraction (%)	58 ± 1	60 ± 1
E peak filling rate (ml/s)	490 ± 21	426 ± 14*
E peak deceleration (ml/s^2 × 10^{-3})	4.4 ± 0.3	3.6 ± 0.2*
A peak filling rate (ml/s)	402 ± 9	401 ± 10
E/A	1.24 ± 0.06	1.08 ± 0.04*
E/Ea	9.1 ± 0.6	10.0 ± 0.7

Data are mean ± standard error or median (interquartile range). *$p < 0.05$ compared with healthy subjects.

A = atrial contraction; E = early diastolic filling phase; E/A = ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate; E/Ea = estimation of left ventricular filling pressures; LV = left ventricular; LV mass/volume ratio = left ventricular mass/left ventricular end-diastolic volume; T2DM = type 2 diabetes mellitus.

0.65 ± 0.05%), as was hepatic triglyceride content (8.6% [2.7% to 24.3%] vs. 2.2% [1.2% to 3.8%], $p < 0.05$) (Table 2, Fig. 1). Myocardial triglyceride content showed significant univariate correlations with age, visceral adipose tissue volume, plasma triglycerides, plasma high-density lipoprotein cholesterol, plasma glucose, plasma insulin concentrations, and hepatic triglyceride content (Pearson r = 0.28, 0.36, 0.37, −0.39, 0.45, 0.30, and 0.37, respectively, $p < 0.05$), but not with BMI, in all study subjects pooled. Visceral adipose tissue volume, plasma high-density lipoprotein cholesterol and glucose concentrations, as well as hepatic triglyceride content remained significantly correlated to myocardial triglyceride content after adjustment for diabetic state; the association between plasma triglyceride levels and myocardial triglyceride content showed borderline significance when adjusted for diabetic state ($p = 0.051$).

Association between myocardial steatosis and myocardial function. Differences in cardiovascular function between T2DM patients and healthy volunteers are displayed in Table 2. The LV diastolic parameters were significantly impaired in T2DM patients, but LV systolic function was not significantly different between healthy subjects and T2DM patients (ejection fraction = 58 ± 1% vs. 60 ± 1%, $p > 0.05$).

E/A and E peak deceleration were used as parameters of LV diastolic function for further analysis. Table 3 lists Pearson correlations of E/A and the E deceleration peak with several parameters in all study subjects and shows that both parameters were significantly inversely correlated to age, heart rate, blood pressure, plasma glucose levels, and myocardial triglyceride content ($p < 0.05$) (Fig. 2).

Multivariable analysis was performed in all subjects to study the association between diastolic function and myocardial triglyceride content. To this purpose, E/A was entered as a dependent variable, and subsequently, myocardial triglyceride content, the presence of T2DM, age, heart rate, and diastolic blood pressure were entered into the model as independent variables (Table 4). Diabetic state had no effect on the association between E/A and myocardial triglyceride content. Furthermore, adjustment for age, heart rate, and diastolic blood pressure, which were all significantly correlated to E/A, had no effect on the association between E/A and myocardial triglyceride content. Identical analyses were performed with E peak deceleration as independent variable. This analysis indicated myocardial triglyceride content associated with E peak deceleration, independently of diabetic state, age, heart rate, and diastolic blood pressure.

Discussion

In this study, we showed that accumulation of myocardial triglycerides in T2DM patients is associated with LV diastolic dysfunction, independently of age, BMI, heart rate, visceral fat, and diastolic blood pressure.

We were able to extend the findings in previous studies showing myocardial steatosis in T2DM patients (4,10).

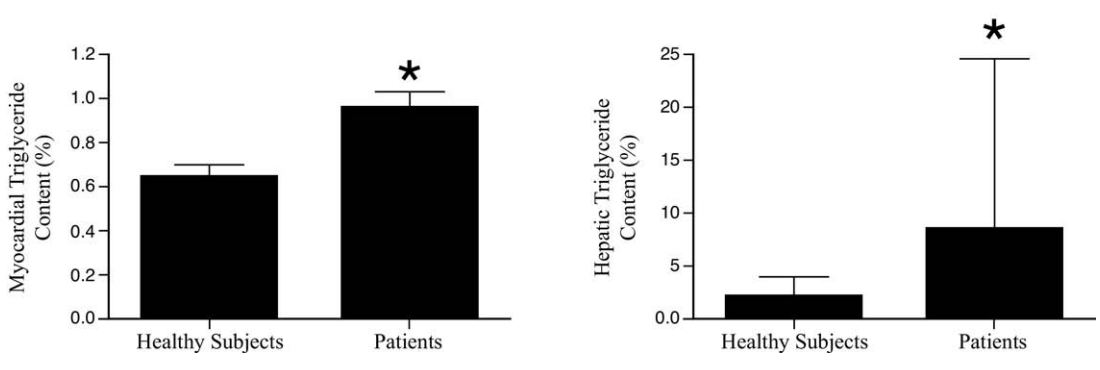

Figure 1 Myocardial and Hepatic Triglyceride Content in Patients and Controls

Bar graphs show increased myocardial and hepatic triglyceride content in diabetic patients as compared with healthy control subjects.
Bars represent mean ± standard error for myocardial triglycerides and median ± interquartile range for hepatic triglycerides. *$p < 0.05$.

Table 3 Univariate Correlations Between Diastolic Function and Anthropometric and Biochemical Markers and Fat Compartments

	E/A	E Peak Deceleration ($ml/s^2 \times 10^{-3}$)
Age (yrs)	−0.58*	−0.44*
Body mass index (kg/m^2)	−0.25*	−0.18
Log heart rate (beats/min)	−0.36*	−0.36*
Systolic blood pressure (mm Hg)	−0.26*	−0.24
Diastolic blood pressure (mm Hg)	−0.41*	−0.45*
LV mass/volume ratio (g/ml)	−0.35*	−0.46*
Plasma glucose (mmol/l)	−0.32*	−0.34*
Log plasma triglyceride (mmol/l)	0.01	−0.05
Nonesterified fatty acids (mmol/l)	0.01	0.09
High-density lipoprotein cholesterol (mmol/l)	0.21	0.28*
Visceral fat (ml)	−0.31*	−0.20
Hepatic triglyceride content (%)	−0.30*	−0.21
Myocardial triglyceride content (%)	−0.42*	−0.40*

Values are Pearson *r*. *$p < 0.05$.
Abbreviations as in Table 2.

McGavock et al. (10) demonstrated that excessive triglyceride accumulation in human cardiomyocytes occurs early in the natural history of T2DM. In their study, a heterogeneous group of T2DM patients showed increased myocardial triglyceride content compared with healthy subjects. The use of insulin (a well-known lipogenic agent, which might have increased myocardial triglyceride levels) by the T2DM patients, and differences in age and BMI between the two groups could have influenced their observations. In addition, the occurrence of silent ischemia, which can be present in up to 22% of asymptomatic T2DM patients with a normal electrocardiogram (21), could influence accumulation of triglycerides in the reversibly injured myocardium during reperfusion (22,23). Therefore, in the present study, we used controls of the same sex and within the same range of age and BMI as patients, and included only T2DM patients with normal dobutamine stress echocardiography to control for reversible ischemia. By using these well-defined groups we could confirm that myocardial triglyceride content in T2DM patients is significantly elevated in comparison with healthy volunteers, independently of age or BMI.

In our study, diabetic patients had higher plasma triglyceride levels and showed significantly lower levels of high-density lipoprotein cholesterol compared with control subjects. Interestingly, plasma triglyceride concentrations were positively associated with myocardial triglyceride content. In addition, we found an inverse correlation between myocardial triglyceride content and high-density lipoprotein cholesterol levels, adjusted for diabetic state. These data are in line with earlier observations demonstrating that increased serum triglyceride and decreased serum high-density lipoprotein cholesterol concentrations correlate with lipid content in skeletal muscle in patients with human immunodeficiency virus–lipodystrophy (24). High-density lipoprotein cholesterol is considered an important mediator of reverse cholesterol transport, a process that involves the transfer and uptake of free cholesterol from the peripheral tissues, with subsequent delivery to the liver, where it can be eliminated. Artificial elevation of high-density lipoprotein in cholesterol-fed rabbits induced the regression of early aortic fatty streaks (25). Based on our results, we hypothesize that reverse cholesterol transport might also play a role in protecting the heart from accumulating lipids.

In the present study, we are the first to demonstrate that myocardial triglyceride accumulation in patients with uncomplicated T2DM is associated with LV diastolic dysfunction, independently of age, BMI, blood pressure, and heart rate. Neutral triglycerides are probably inert and harmless to cells and may initially even provide a protective buffer by diverting NEFA from deleterious pathways (26). Eventually, however, excessive triglyceride stores enter a continuous cycle between hydrolysis and fatty acid re-esterification, yielding cardiotoxic intermediates, such as ceramide and

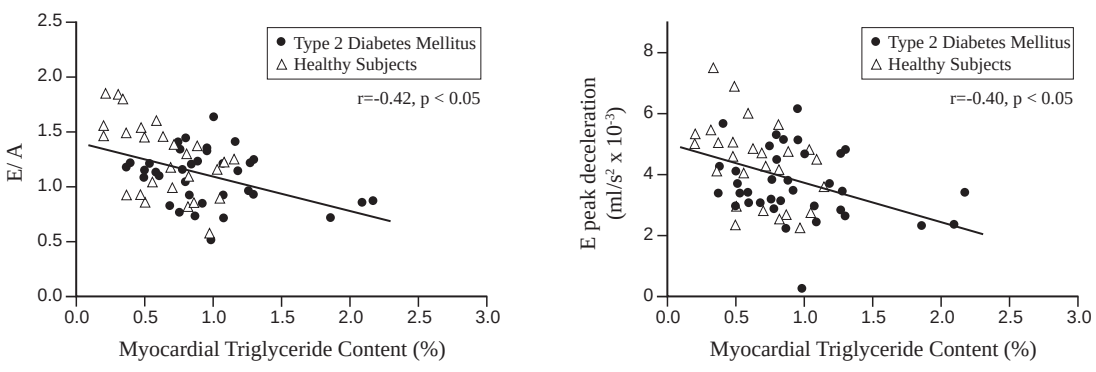

Figure 2 Correlations Between Myocardial Triglyceride Content and Left Ventricular Diastolic Function

Increased myocardial triglyceride content is significantly associated with decreased myocardial function.
E = early diastolic filling phase, E/A = ratio of maximal left ventricular early peak filling rate and the maximal left ventricular atrial peak filling rate.

Table 4 Multivariable Associations Between E/A and Myocardial Triglyceride Content

	E/A Beta (95% CI)	p Value*	r^2	p Value†
Model 1			0.18	<0.001
Myocardial triglyceride content (%)	−0.31 (−0.48 to −0.15)	<0.001		
Model 2 (Model 1 + diabetic state)			0.19	0.001
Myocardial triglyceride content (%)	−0.28 (−0.46 to −0.10)	0.003		
T2DM/healthy subject	0.07 (−0.07 to 0.22)	0.32		
Model 3 (Model 2 + age)			0.42	<0.001
Myocardial triglyceride content (%)	−0.20 (−0.36 to −0.04)	0.015		
T2DM/healthy subject	0.02 (−0.10 to 0.15)	0.725		
Age (yrs)	−0.02 (−0.03 to −0.01)	<0.001		
Model 4 (Model 3 + heart rate)			0.46	<0.001
Myocardial triglyceride content (%)	−0.20 (−0.35 to −0..04)	0.014		
T2DM/healthy subject	−0.03 (−0.16 to 0.10)	0.671		
Age (yrs)	−0.02 (−0.03 to −0.01)	<0.001		
10% increase in heart rate (beats/min)	−0.97 (−1.81 to −0.14)	0.023		
Model 5 (Model 4 + diastolic blood pressure)			0.50	<0.001
Myocardial triglyceride content (%)	−0.20 (−0.35 to −0.05)	0.009		
T2DM/healthy subject	−0.05 (−0.17 to 0.08)	0.487		
Age (yrs)	−0.02 (−0.03 to −0.01)	<0.001		
10% increase in heart rate (beats/min)	−0.89 (−1.71 to −0.08)	0.032		
Diastolic blood pressure (mm Hg)	−0.01 (−0.02 to −0.001)	0.031		
Model 6 (Model 3)				
Association between E/A and myocardial triglyceride content after adjusting for diabetic state, age, *and* each of following variables				
a. LV mass/volume ratio	−0.19 (−0.34 to −0.03)	0.017	0.47	<0.001
b. Visceral fat (ml)	−0.19 (−0.35 to −0.02)	0.026	0.42	<0.001
c. 10% increase in hepatic triglyceride content	−0.20 (−0.38 to −0.01)	0.036	0.42	<0.001
d. Fasting plasma glucose (mmol/l)	−0.19 (−0.36 to −0.02)	0.025	0.43	<0.001
e. Body mass index (kg/m^2)	−0.23 (−0.33 to −0.01)	0.033	0.44	<0.001

r^2 for the respective models, that is, in Model 1, with E/A as dependent and myocardial triglyceride content as independent variable; in Model 2, myocardial triglyceride content and diabetic state are independent variables; in Model 3, myocardial triglyceride content, diabetic state, and age are independent variables; in Model 4, myocardial triglyceride content, diabetic state, age, and heart rate are independent variables; in Model 5, myocardial triglyceride content, age, diabetic state, heart rate, and diastolic blood pressure are independent variables; in model 6 (a to e), the possible confounders are separately entered for adjustment and beta and p values for the association between myocardial triglyceride content and E/A are displayed. *Level of significance for the association between E/A and the separate components of the model; †level of significance of the model.

CI = confidence interval; other abbreviations as in Table 2.

diacylglycerol, which seems to be an important route leading to myocardial dysfunction, at least in animal models (7,27).

In the present study, LV ejection fraction was not different between patients and healthy control subjects and was dissociated from myocardial triglyceride content. We excluded patients with myocardial ischemia and contractile abnormalities, using dobutamine stress echocardiography, but microvascular disease cannot be ruled out. Previous experimental studies (28) have shown decreased contractility and diastolic function contemporary with hypertrophy and concentric remodeling. In our patient population with uncomplicated diabetes mellitus, cardiac mass was not different from that in healthy volunteers. LV mass/volume ratio was increased in T2DM patients, indicating mild concentric remodeling (29). Therefore, LV mass/volume ratio was included in the multivariable analysis, but had no influence on the independent relationship between myocardial triglyceride content and diastolic function.

Based on these findings, we hypothesize that there may be a disease course starting with lipid accumulation in the myocardium, leading to diastolic dysfunction. In a later stage of disease, global systolic function may be impaired, as diastolic abnormalities are most commonly observed earlier than systolic abnormalities (30).

Furthermore, in uncomplicated T2DM, visceral fat volume, and hepatic triglyceride content were increased and correlated to myocardial triglyceride content after adjustment for diabetic state. Our findings support the evidence that lipotoxic processes in cardiomyocytes constitute an important mechanism underlying the epidemiologic association between visceral adiposity, ectopic steatosis, and cardiovascular disease (10,31,32).

In the present study design, we cannot establish a causal relationship between increased myocardial triglyceride content and reduced LV function. We cannot discriminate if myocardial triglyceride accumulation per se hampers myocardial function due to a mechanical effect, the formation of cardiotoxic intermediates, or by intervening in other mechanisms such as calcium handling. In addition, excluding women from the study limits the generalizability of the present study. Further studies need to be initiated to make these distinctions and to extend our findings to female

subjects to identify the role of myocardial triglyceride accumulation in the human diabetic heart, because in animal models, therapeutic interventions aiming at reduction of myocardial triglyceride accumulation due to disturbed fatty acid metabolism have been shown to have beneficial effects on myocardial function (7).

Furthermore, pioglitazone, a peroxisome proliferator-activated receptor gamma agonist, which has the capacity to divert fat from nonadipose tissue to subcutaneous tissue, has been shown to lower myocardial triglyceride content in T2DM patients on an insulin-based treatment regimen (33). Therefore, myocardial triglyceride content might be a useful indicator of myocardial steatosis for predicting the severity of diabetic cardiomyopathy and for evaluating the effects of antisteatotic therapy.

Conclusions

Myocardial triglyceride content is increased in uncomplicated T2DM and associated with impaired LV diastolic function, independently of age, BMI, heart rate, visceral fat, and diastolic blood pressure. Therefore, myocardial steatosis might be a useful indicator for predicting the severity of diabetic cardiomyopathy and for evaluating the effects of antisteatotic therapy.

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