The myocardium of patients with type 2 diabetes mellitus (T2D) loses its metabolic flexibility because of elevated circulating free fatty acid (FFA) levels and myocardial lipid content (MyLC). This creates a state of lipotoxicity that compromises the contractile function and promotes heart failure (HF). These processes are reversible by interventions that reduce FFA fluxes. MyLC, as measured by magnetic resonance proton spectroscopy (MRS), is increased in patients with T2D and normal left ventricular ejection fraction (LVEF). These observations suggest that short-term suppression of circulating lipid may have adverse cardiac effects in patients with symptomatic HF, especially in patients with elevated lipid levels such as in T2D. However, the impact of high as opposed to low circulating levels of FFA on MyLC and cardiac function in patients with T2D and HF is unknown.

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We hypothesized that (1) short-term extreme changes in FFA and triglyceride levels affect MyLC in patients with T2D and HF, and that (2) suppressed as opposed to increased circulating FFA and triglyceride increase myocardial contractility and cardiopulmonary exercise capacity. The aim of the present study was to investigate the relationship among circulating lipid levels, MyLC, and cardiac function together with the acute cardiac effects of high as opposed to low circulating free fatty acid (FFA) and triglyceride levels in patients with type 2 diabetes mellitus and heart failure.
study was hence to investigate the impact of short-term manipulation of circulating FFA and triglycerides on MyLC, cardiac systolic and diastolic function, and stress-induced contractile reserve, and the correlation between changes in MyLC and contractile function.

Patients with T2D and HF were included in a randomized crossover–designed trial. They underwent 8 hours of intralipid/heparin infusion (high FFA) and hyperinsulinemic-euglycemic clamping (low FFA). We used advanced echocardiography, cardiopulmonary exercise testing, and MRI to measure cardiovascular function and MRS to quantify MyLC.

Methods

Patients

We included 20 noninsulin-treated white male patients with T2D and chronic HF. The patients were stable on optimal HF medication, in New York Heart Association class 2 to 3 and had a LVEF ≤54% assessed by echocardiography. We excluded patients with high age (>85 years), cardiac valve disease, physical or psychological disability, creatinine >220 mmol/L, alanine aminotransferase >3-fold the upper normal limit, or myocardial infarction within the past 3 months. MyLC was measured in the interventricular septum by MRI. Thus, we excluded patients with large septal scars visualized by echocardiography.

Design

All patients were investigated in a randomized crossover–designed trial on 2 different occasions 1 to 6 weeks apart. They were assigned by the investigators to either high FFA followed by low FFA or the opposite (one to one) by drawing sealed envelopes. Patients were studied in the fasting state after 8 hours of hyperinsulinemic-euglycemic clamping at plasma glucose of 5 mmol/L using 0.8 mIU insulin/kg per minute. (Actrapid, Novo Nordisk, A/S, Denmark; low FFA) and after 8 hours of infusion of Intralipid 20% (Fresenius Kabi AB, Uppsala, Sweden) at 40 mL/h combined with 250 U/h of heparin (high FFA). To avoid bias from other antiidiabetics, we chose to instruct all participants to pause oral antiidiabetic medication 2 days before the investigations. Patients were admitted at 7:45 AM and venous cannulas were inserted into each of the upper extremities for infusion and blood sampling. Infusions were initiated at 8:15 AM (60 hours) and continued throughout the day of investigation. In the low-FFA arm, plasma glucose concentration was measured every 10 minutes, and isotonic saline was administered with an individual variable 20% glucose infusion to avoid hypoglycemia. In the high-FFA arm, isotonic saline was coadministered with intralipid/heparin infusion and plasma glucose was measured every second hour. On both occasions, blood samples for analysis were drawn again at t=6 hours, echocardiography was performed at t=7 hours, cardiopulmonary exercise testing at t=8 hours, and myocardial MRI followed by MRS of the heart between t=9 and t=10 hours. We were unable to repeatedly perform plasma glucose measurements in the MR scanner room. Thus, the hyperinsulinemic-euglycemic clamp was terminated before the MR investigations for safety reasons. The intralipid/heparin infusion was continued until completion of the MR investigations.

Post processing of the data was blinded to the investigators.

Blood Samples

Plasma glucose measurements were performed using HemoCue Glucose 201 (Angelholm, Sweden), which measures whole blood glucose and calculates plasma glucose. Additional blood samples were immediately cooled, spun, and stored at −80°C until analysis. Blood samples were analyzed for levels of FFA, insulin, C-peptide, glucagon, cortisol, growth hormone (GH), insulin-like growth factor 1, and adiponectin as previously described. Adrenaline and noradrenaline were measured by a valid in-house time-resolved high-performance liquid chromatography method, β-hydroxybutyrate by a commercial amperometric method (Randox analysis kit RB1007, Abbott), and N-terminal probrain natriuretic peptide using a commercial electrochemiluminescent assay (Roche Diagnostics, Denmark).

Echocardiography

Echocardiography was performed by a single operator using a Vivid Seven ultrasound scanner (GE Medical System, Horten, Norway) with a 2.5-MHz transducer. Sonovue (Branco, Initios Medical AB, Copenhagen, Denmark) was administered intravenously as previously described to enhance the left ventricular endocardial border delineation. EchoPAC 11 software (GE-Vingmed Ultrasound, Horten, Norway) was used for analysis. LVEF was measured using the biplane-modified Simpson method. Peak systolic longitudinal mitral plane velocities during ejection phase (Smax) were measured by tissue Doppler imaging and global strain by 2D speckle tracking as previously described. Measurements were performed at rest and immediately after peak exercise. We assessed left ventricular diastolic function from mitral inflow and tissue Doppler: E/A-ratio, E-deceleration time, isovolumetric relaxation time, and mitral plane e′ (early diastolic) velocity. Parameters were estimated as averages of either 3 (sinus rhythm) or 5 (atrial fibrillation) consecutive heart beats. All echocardiographic investigations were blinded before analysis.

Resting Hemodynamics

Systolic and diastolic blood pressure was measured in the same arm on both visits, and mean arterial pressure (MAP) was calculated (MAP=pulse pressure/3+diastolic blood pressure).

Exercise Testing

A ZAN600 CPET (nSpire Health GmbH, D-97723 Obergthulba, Germany) was used. Patients performed a staged exercise bicycle test with stages lasting 1 minute and increments of 10 W/min. Blood pressure, heart rate, and ECG were measured repeatedly every second minute. Oxygen consumption and carbon dioxide excretion were measured continuously at rest and during the exercise test as described by the manufacturer.

Magnetic Resonance Investigation

A Philips 1.5 Tesla scanner and a 5-element cardiac coil were used. All MRI measurements were ECG-triggered and breath-hold was applied. MRI of the 4-chamber, the 2-chamber, and 12 slices with the biplane-modified Simpson method. Peak systolic longitudinal (Smax) and LVEF were measured continuously at rest and during the exercise test as described by the manufacturer.

Primary end points were defined as changes in MyLC. Secondary end points were changes in LVEF; myocardial contractile function (Smax and global strain), peak exercise capacity, oxygen consumption, postexercise LVEF, and contractile function. The outcomes were measured consecutively and analyzed after completion of the last patient.

Statistics

We applied D’Agostino and Pearson omnibus normality test. E/A-ratio (rest, P value=0.02; exercise, P value <0.001) and time from
last coronary angiography \( (P=0.02) \) did not pass this normality test and are, therefore, presented as median (25%–75% percentile). All other values are presented as mean±SD. \( P \) values <0.05 were considered significant. Paired \( z \) test or Wilcoxon signed rank test was used to analyze data between study arms. Spearman was used to test for correlations. Two-way ANOVA with repeated measurements and robust clustered variance analysis were used when stated. Confidence intervals of 95% are presented when appropriate. On the basis of prior or studies, a crossover design with 20 patients, an expected drop out of 10%, a significance levels of 5%, and a power of 80%, we expected to be able to detect changes in left ventricular contractility (S\(_\text{max}\), strain) of 6% (in relative percentage) and LVEF of 4% (in absolute percentage). The coefficient of variation of myocardial MRS in healthy subject is 18%. Applying these parameters and complete data sets on ≥10 patients, we would expect to be able to detect differences of 18% in MyLC.

### Ethics

Data were collected according to the protocol at the Department of Cardiology, Department of Endocrinology and Metabolism, and the MR-Center at Aarhus University Hospital, Aarhus, Denmark. The protocol was approved by the Central Denmark Region Committee on Health Research Ethics and informed written consent was obtained from each patient.

### Results

#### Patients

Forty-three patients from our outpatient HF clinic were eligible and they were screened for inclusion between 2010 and 2011. Twenty-three were excluded either because they did not meet the inclusion criteria \( (n=21) \) or because they met the exclusion criteria according the protocol \( (n=2) \). Twenty patients were enrolled. One experienced cardiac arrest before the first visit (allocated to high FFA followed by low FFA) and another did not attend the first visit and withdrew consent (allocated to low FFA followed by high FFA). Eighteen patients completed echocardiography and exercise and postexercise echocardiography on both occasions. Patients were aged 67±7 years, had an LVEF of 35±8%, and were in New York Heart Association class 2 or 3. The HF symptoms were because of either ischemic heart disease \( (n=15) \) or dilated cardiomyopathy \( (n=3) \). Two patients had atrial fibrillation as comorbidity. The mean duration of T2D was 5±4 years and HbA1c was 6.8±1.5%. All patients were on optimal medical HF treatment. Fourteen patients were on oral antidiabetic treatment and 4 patients on dietary treatment. Twelve patients completed the MRI and 10 patients completed cardiac MRS on both occasions. Dropouts from the MR analysis were because of patients having a pacemaker \( (n=3) \), claustrophobia \( (n=2) \) during the scan, incomplete cardiac MR data \( (n=1) \), or technical problems during the myocardial MRS \( (n=2) \). No coronary angiography was performed before enrollment. However, all but 1 patient was subjected to angiography within the past 3 years \( (7 \text{[5–13] months}; \text{median, 25%–75%}) \) which included or documented full revascularization of the arteries supplying the septum. The last patient underwent coronary angiography 5 years before enrollment and was diagnosed with dilated cardiomyopathy (Table 1; Figure 1).

#### Substrates and Hormones

FFA levels differed >20-fold \( (0.05±0.04 \text{ mmol/L [low FFA]} \text{ versus } 1.04±0.27 \text{ mmol/L [high FFA]; } P<0.001) \) and triglycerides

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<th>Table 1. Patient Characteristics</th>
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<td><strong>Age, y</strong></td>
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<td><strong>NYHA 2/NYHA 3 (n)</strong></td>
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<td><strong>IHD (n)</strong></td>
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<td><strong>DCM (n)</strong></td>
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<td><strong>Atrial fibrillation (n)</strong></td>
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<td><strong>LVEF, %</strong></td>
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<td><strong>Duration of diabetes mellitus, y</strong></td>
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<td><strong>Weight, kg</strong></td>
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<td><strong>HbA1c, %</strong></td>
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<td><strong>C-Peptide, pmol/L</strong></td>
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<td><strong>FFA, mmol/L</strong></td>
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<td><strong>Triglycerides, mmol/L</strong></td>
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**Medication/treatment**

| ICD or CRT system, n | 3 |
| ACE inhibitors, n | 18 |
| \( \beta \)-Blockers, n | 17 |
| Spironolactone, n | 2 |
| Other antihypertensive therapy, n | 4 |
| Acetylsalicylic acid, n | 18 |
| Metformin, n | 12 |
| Sulfonylurea, n | 4 |
| DPP4 inhibitors, n | 2 |
| Diet (as only treatment), n | 4 |

ACE indicates angiotensin-converting enzyme; BP, blood pressure; CRT, cardiac resynchronization therapy; DCM, dilated cardiomyopathy; DPP4, dipeptidyl peptidase-4; FFA, free fatty acid; ICD, implantable cardioverter-defibrillator; IHD, ischemic heart disease; LVEF, left ventricular ejection fraction; and NYHA, New York Heart Association classification.
MR Spectroscopy

MyLC differed by 50% between the study arms (0.78±0.59% versus 1.16±0.73%; P=0.006; Figure 2). LVEF and MyLC displayed an inverse relation (r=-0.54; r²=0.29; P=0.004; Figure 3). MyLC correlated positively with FFA (r=0.45; r²=0.20; P=0.001; Figure 4A) and triglycerides (r=0.47; r²=0.22; P=0.003; Figure 4B). However, these 2 correlation did not differ (P=0.08). We identified an outlier with regard to the triglyceride levels by Grubb test. Including these data did not affect the level of significance. MyLC and insulin were negatively associated (r=-0.32; r²=0.10; P=0.04; Figure 4C). The homeostasis model assessment index, C-peptide, glucose, HbA1c, body mass index, age, and N-terminal probrain natriuretic peptide levels did not correlate with MyLC (data not shown).

Left Ventricular Function, Contractility, and Resting Hemodynamics

No differences between the study arms were observed in LVEF (ΔLVEF=1±6%; confidence interval, −4% to 2%; P=0.60 [echocardiography] and ΔLVEF=1±4%; confidence interval, −3% to 2%; P=0.64 [MRI]), strain, or cardiac output. S’max was higher during high FFA than during low FFA, and MAP was highest during high FFA. Diastolic parameters did not differ between high and low FFA (Table 2).
Exercise Test, Hemodynamics, and Postexercise Echocardiography
Exercise capacity, MAP, peak O₂ consumption, LVEF, S’max, and strain post exercise did not differ between study arms and neither did the diastolic parameters. The respiratory equation ratio was significantly higher during rest and peak exercise in the high-FFA arm than in the low-FFA arm. Because of the placement of the ROI during MRS, we measured regional septal strain from rest to exercise and found it to increase (P=0.02; 2-way ANOVA with repeated measurements; Table 2).

Discussion
In patients with T2D and HF, MyLC displayed much flexibility in response to variations in circulating lipids and correlated inversely with LVEF and positively with FFA and triglyceride levels. Short-term elevated as opposed to suppressed FFA and triglyceride levels had no detrimental effect on LVEF, strain, exercise-induced contractile reserve, or cardiopulmonary exercise capacity. In contrast, a minor increase in S’max was observed during high lipid levels. These findings suggest that short-term lowering of circulating lipid levels and MyLC has no overt beneficial effect on left ventricular function.

Short-term Modulation of Circulating FFA in Patients With T2D and HF
FFA and myocardial lipid accumulation have been implicated in the progression of HF. The present study addresses the clinical and functional effects of extreme differences in FFA and triglycerides in patients with T2D, reduced LVEF, and symptomatic HF. Studies in HF patients without T2D have shown either neutral or even potentially detrimental effects of suppression in circulating FFA levels on left ventricular function and cardiac efficiency. These discordant results may reflect differences in the pathophysiology of patients with and without diabetes mellitus and differences in the severity of the metabolic derangement such patients undergo. The FFA levels differed >20-fold and triglyceride levels 4-fold between the study arms. Even so, we detected no significant deterioration in our end points of LVEF, cardiac output, strain, diastolic function, contractile reserve, or cardiopulmonary exercise capacity during high FFA as opposed to low FFA. In contrast, we observed a minor, but significant increase in S’max in patients with high circulating levels of FFA and triglycerides. This could be because of the fact that S’max is more reproducible and more sensitive to subtle contractile changes than LVEF, even by contrast-enhanced echocardiography. Furthermore, the present changes in S’max may have clinical relevance as it has been shown to be an independent predictor of mortality.

Our findings seem unlikely to be caused by changes in after or preload. We would have expected the increase in MAP (and hence afterload) during high FFA to decrease S’max, but the opposite was observed. Although weight and plasma volume did not differ significantly between the study arms, more fluid tended to be infused during low FFA (<140 mL; near-significant [P=0.06]) than during high FFA. This could potentially have decreased the differences in S’max between the study arms as a volume challenge increases S’max in patients with cardiac disease. However, the difference in volume was negligible. Furthermore, we would have expected these potential differences in volume load, if present, to affect preload and thus e’. However, this was not observed. Our finding seems not to be explained by a confounding effect of insulin stimulation on the sympathetic nervous system because insulin levels were lower during high FFA than during low FFA, the heart rate was unchanged, and catecholamine levels did not differ between the study arms. Furthermore, no evidence...
supports that GH or glucagon, which differed between the study arms, could have caused the observed effects in MyLC or $S^{\prime \max}$. However, differences in circulating GH concentrations could have diminished the difference in $S^{\prime \max}$ as GH increases myocardial contractility. C-peptide differed between the study arms, but increased C-peptide levels have no vascular effects during preserved endogenous C-peptide production. Because all patients in the present study had preserved endogenous C-peptide production, it is unlikely that different C-peptide levels biased our results. This suggests that the observed differences in $S^{\prime \max}$ between the study arms may have been caused by intrinsic myocardial metabolic changes. Our findings supplement the observations by Tuunanen et al, who reported that both glucose and FFA metabolism in stable HF patients does not decrease LVEF, but on the contrary it increases stroke volume and $S^{\prime \max}$. A potential explanation of this observation may be that in terms of ATP yield, thus, the decrease in cardiac fatty acid oxidation during low circulating lipid levels is not compensated for by the increase in glucose oxidation. Whether the discrepancies between Tuunanen et al and the present findings are rooted in differences with regard to T2D and the applied interventions are unknown. However, further studies are required to elucidate whether the failing heart responds differently to more modest reductions in circulating lipids, as previously stated. Although the changes in circulating substrates were extreme in our study, the clinical significance of decreased $S^{\prime \max}$ during hyperinsulinemic-euglycemic clamping and increased contractile function during hyperglycemia and elevated FFA as previously shown could suggest that a major decrease in lipid load may be detrimental in patients with T2D and HF.

**Short-term Modulation of MyLC in Patients With T2D and HF**

Observational studies in patients with T2D and normal LVEF demonstrate that elevated MyLC correlates inversely with diastolic function and systolic strain. MyLC measured in myocardial biopsies is also elevated in patients with reduced LVEF because of aortic stenosis and end-stage dilated cardiomyopathy, and it is even more elevated in case of coexisting T2D. Although we were unable to detect differences in LVEF and exercise capacity between high and low circulating FFA concentrations, we demonstrated an inverse correlation between LVEF and MyLC. However, it is unknown whether this correlation reflects causality. The ability to accumulate MyLC in HF may serve as a protective buffer against detrimental lipid intermediates, it may give rise to decreased energy metabolism and accumulation of toxic lipid intermediates, or it may merely constitute an energy store when excess FFA is taken up by the cardiomyocyte. Thus, this finding of an inverse correlation between LVEF and MyLC needs to be addressed in future long-term studies. However, such studies should be carefully monitored as we observed a small, but significant decrease in $S^{\prime \max}$ during reduced MyLC.

In the present study, the extreme differences in circulating FFA and triglyceride levels caused a difference of 50% in MyLC between the study arms. This indicates that MyLC
remains flexible to short-term extreme changes in whole-body substrate supply in patients with T2D and HF. This magnitude of change in MyLC is similar or even greater than that observed in previous studies of non-HF patients, and it is in accordance with findings of a high myocardial FFA uptake in case of impaired glucose tolerance.

FFA, triglyceride levels, and MyLC correlated positively. In contrast, a previous study showed that MyLC did not increase because of elevated triglycerides 4 hours after oral lipid intake in normal test subjects. However, recently it was shown that in patients with impaired glucose tolerance as compared with healthy test subjects, postprandial myocardial lipid uptake is increased, although FFA levels decrease while triglyceride levels increase. This suggests that results on myocardial lipid metabolism obtained in healthy subjects are not readily transferred to patients with metabolic derangement.

The present study shows that the metabolic disturbances have not reached an end stage that eliminates the flexibility of lipid storage in patients with T2D and HF. Thus, the failing heart of patients with T2D has preserved its ability to sequester lipid without impeding the myocardial contractile function or causing clinically significant acute lipotoxicity in patients with T2D and HF.

Study Limitations

Plasma glucose was increased during high FFA. It can be argued that this might cause a minor increase in myocardial glucose uptake because of a glucose mass effect. However, this seems unlikely because FFA differed >20-fold between the study arms and circulating FFA levels correlate inversely with myocardial glucose uptake. Similarly, ketones differed between the study arms, but to our knowledge, no human experiments support that this should affect our results.

Winhofer et al report that hyperglycemic clamp and hyperinsulinemia cause an increase in MyLC in normal subjects. We found that MyLC was lowest during hyperinsulinemic-euglycemic clamping (low FFA). We suggest that this discrepancy is because of the large differences in triglycerides and FFA levels between our study arms.

The effect of high lipid levels on insulin sensitivity and hyperinsulinemia was not investigated but could have been addressed in a third study arm by combining high FFA levels with hyperinsulinemic-euglycemic clamping (low FFA). We suggest that this discrepancy is because of the large differences in triglycerides and FFA levels between our study arms.

Conclusion

The failing heart of patients with T2D can adapt to extreme short-term increases in circulating lipids and does not display features of acute myocardial lipotoxicity. The long-term cardiac effects of modulating myocardial lipid uptake and content await further studies.

Acknowledgments

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Disclosures

Dr Schär is employed by Philips. The other authors report no conflict.

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annular velocity measured with Doppler tissue imaging in patients with chronic heart failure caused by left ventricular systolic dysfunction. *Heart*. 2006;92:775–779.


**CLINICAL PERSPECTIVE**

This clinical study addresses the cardiovascular effects of manipulating circulating lipid levels and myocardial lipid content in patients with type 2 diabetes mellitus and heart failure. Thus, it contributes to the understanding of cardiac lipotoxicity and the clinical approach toward patients with type 2 diabetes mellitus and heart failure. Our study shows that myocardial lipid content is inversely correlated with left ventricular ejection fraction and displays much flexibility in response to changes in circulating triglycerides, free fatty acids, and insulin levels. However, short-term suppression of circulating lipid levels and myocardial lipid content has no beneficial effect on the failing diabetic myocardium. However, we observed that this may even have adverse effects with regard to cardiac contractile function. For the practicing clinician, the present study, therefore, shows that reduction of myocardial lipid content through brief suppression in circulating lipids is without beneficial clinical effects in patients with heart failure and type 2 diabetes mellitus. These are new findings in this large subgroup of heart failure patients with a poor prognosis.
Failing Heart of Patients With Type 2 Diabetes Mellitus Can Adapt to Extreme Short-term Increases in Circulating Lipids and Does Not Display Features of Acute Myocardial Lipotoxicity

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