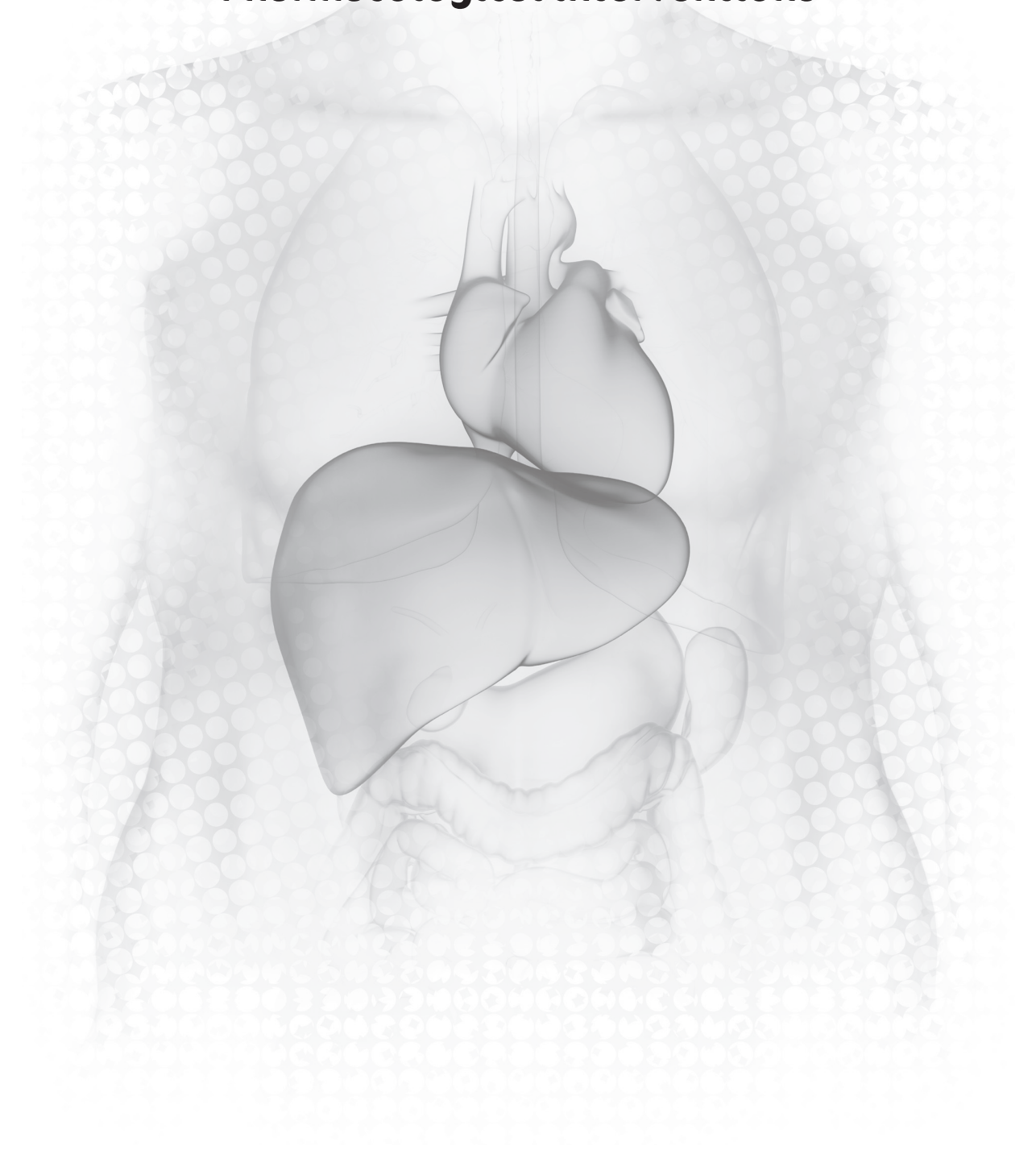
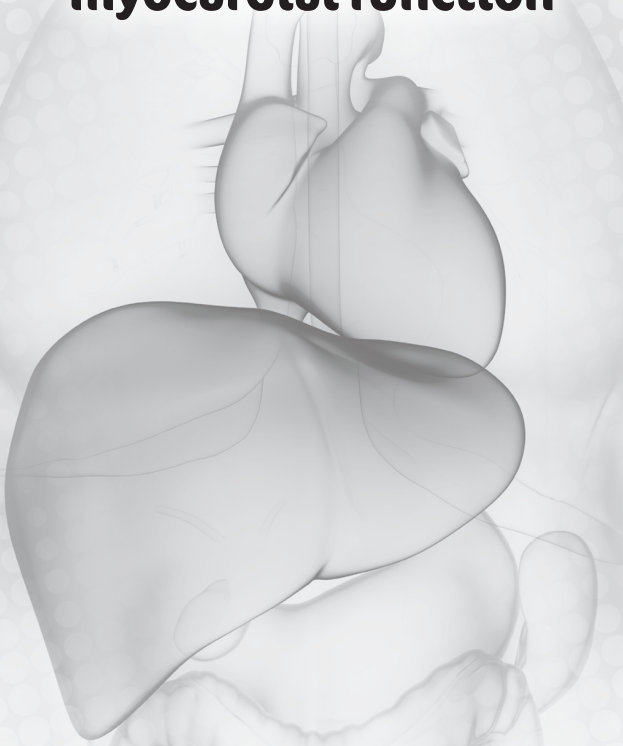
PART III

Pharmacological interventions



Chapter 10

Short-term hyperglycemic dysregulation in patients with type 1 diabetes does not change myocardial triglyceride content or myocardial function



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Diabetes Care 2008; 31:1613-1614

ABSTRACT

Background

Patients with type 1 diabetes (T1DM) suffer from frequent episodes of hyperglycemia and high plasma levels of non-esterified fatty acids (NEFA) despite insulin treatment. The aim of this study was to evaluate the effects of hyperglycemia due to partial insulin deprivation on myocardial triglyceride (TG) content and myocardial function in patients with T1DM.

Methods

Myocardial and hepatic TG content and left ventricular (LV) function were measured in 10 patients with T1DM (mean ± SEM HbA1c: 7.4 ± 0.2 %) using proton magnetic resonance (MR) spectroscopy and MR imaging respectively. Subjects were studied during optimal gluco-regulation and after 24 hours of partial insulin deprivation to induce plasma glucose levels between 15 and 20 mmol/L.

Results

Mean insulin infusion rate was 45 ± 5 units at baseline, whereas it was 27 ± 5 units during hyperglycemia (per 24 h, $P<0.001$). During partial insulin deprivation plasma 24 hour glucose levels increased from 8.4 ± 0.6 to 15.9 ± 0.8 mmol/L ($P<0.001$), and plasma NEFA levels from 0.31 ± 0.05 to 0.46 ± 0.07 mmol/L ($P=0.015$). Hyperglycemia had no effects on myocardial TG content (0.31 ± 0.04 and 0.34 ± 0.06%, resp., $P=0.6$) or hepatic TG content and LV function.

Conclusion

Short-term hyperglycemic dysregulation does not modulate myocardial or hepatic TG content or myocardial function, despite considerable metabolic adaptations.

INTRODUCTION

Intensive insulin treatment is the cornerstone of therapy of patients with type 1 diabetes (T1DM). Nonetheless, this treatment is not fully able to restore glucoregulation to normal, and patients with T1DM suffer from frequent episodes of suboptimal metabolic regulation, reflected in short or longer lasting episodes of hypo and hyperglycemia. These problems in optimalization of glucoregulation may also result in altered lipid metabolism. Intramyocellular lipid content is increased in patients with T1DM compared with that in control subjects and is associated with glycated hemoglobin (HbA1c) values (1). Therefore metabolic dysregulation may play a major role in the induction of abnormal intramyocellular lipid accumulation in type 1 diabetes.The metabolic effects of T1DM also extend to the heart. Studies with positron emission tomography showed increased myocardial fatty acid utilization and oxidation in patients with T1DM, whereas myocardial glucose utilization is reduced (2,3). Myocardial substrate metabolism in these patients is influenced by plasma insulin and non-esterified fatty acid (NEFA) levels (4). Therefore, it is likely that cardiac metabolism is also affected by episodes of metabolic dysregulation in patients with type 1 diabetes.

In several conditions there is a discrepancy between fatty acid uptake and fatty acid utilization in the heart, reflected in alterations in myocardial TG content. For instance, we documented that caloric restriction induces a dose-dependent increase in plasma NEFA levels and myocardial TG content in healthy subjects (5). Therefore, myocardial TG content is not fixed, but can be modulated depending on metabolic conditions. Moreover, this increase in myocardial TG content during caloric restriction was associated with impaired diastolic function (5,6). In accordance, in animal experiments increased myocardial TG is associated with impaired myocardial function (7,8), via routes involving fatty acid derivatives (9-11). Therefore, we hypothesized that episodes of metabolic dysregulation in patients with T1DM due to insufficient insulin provision may adversely affect myocardial TG content and myocardial function.

The primary aim of the present study was to evaluate the effects of short-term metabolic dysregulation, caused by insufficient insulin provision, on myocardial TG content and myocardial function in patients with type 1 diabetes, otherwise well controlled by continuous insulin pumps. Recent optimalization of proton (^{1}H) magnetic resonance (MR) spectroscopy techniques allowed us to study myocardial TG content and myocardial function in vivo. To assess the tissue specificity of the potential changes in myocardial TG content, we also assessed hepatic TG content on both occasions.

METHODS

Patients

We studied 10 (mean age ± SEM: 41 ± 11 years), C-peptide negative patients with T1DM (5 men, HbA1c 7.4 ± 0.2 %, Table 1). The sample size was based on our previous experiments in healthy subjects, in which we observed a statistical power of 0.89 for detecting a mean increase in myocardial TG content of 0.23% in 10 subjects (12). The mean duration of diabetes was 22 ± 2 years. All subjects used insulin treatment by insulin pump therapy (continuous subcutaneous insulin infusion) and used frequent self monitoring of blood glucose levels. A screening visit was performed with a medical history, physical examination and routine laboratory tests. Exclusion criteria were: smoking, an abnormal electrocardiogram (ECG), hypertension, use of other medication known to influence lipolysis and/or glucose metabolism (especially thiazolidinediones) or renal (microalbuminuria <30 mg/24h, normal creatinin levels), hepatic or other endocrine disease. Furthermore, obese subjects (BMI > 30 kg/m^2) were excluded.

The experimental protocol was approved by the institutional ethical committee and all subjects signed informed consent prior to participation.

Study design

The study consisted of 2 study occasions separated by a washout period of at least 2 weeks. The baseline study was done after a 3-day period, in which subjects aimed at optimal blood glucose levels measured by a continuous glucose monitoring system (Metronic MiniMed Inc., Northridge, CA, USA). Patients were instructed to document their basal and bolus insulin infusions. The second study was after ~50% reduction in both basal and bolus insulin infusions during 24 hours, compared with the first study, in order to maintain hyperglycemia between 15 and 20 mmol/L. Patients self-measured and documented their plasma glucose levels and ensured blood glucose levels remained < 20 mmol/L during targeted partial insulin deprivation. For both occasions, patients were instructed to maintain the same caloric intake for 3 days before examination. As the study was designed to mimic hyperglycemic dysregulation in daily life, the subjects were instructed to remain their daily routine during both the study periods. Before MR examination, blood samples (postprandial) were taken.

^{1}H-magnetic resonance spectroscopy of the heart and the liver

All MR measurements were performed on a 1.5-Tesla Gyroscan ACS-NT MRI scanner (Philips Medical Systems, Best, The Netherlands) with patients in the supine position at rest. MR data were obtained in the afternoon. For MR spectroscopy measurements, a body coil for radiofrequency transmission and a surface coil for signal receiving were used. A point resolved spatially localized spectroscopic pulse sequence was used to acquire single voxel (8 mL) spectra. The myocardial voxel (2 × 4 × 1 cm) was placed in the myocardial septum on standard four-

chamber and short axis images at end-systole, avoiding contamination with epicardial fat. Data acquisition was double-triggered using ECG triggering and navigator echoes, to minimize breathing artifacts (12). For the liver, voxel sites were matched at the study occasions (by using the twelfth thoracic vertebra as an anatomical landmark), avoiding vascular structures and bile ducts. Water-suppressed spectra with 128 averages were collected to detect weak lipid signals from the heart, and suppressed spectra with 64 averages were acquired from the liver. Spectral parameters included: repetition time (TR) of at least 3000 ms, and an echo time (TE) of 26 ms. 1024 Data points were collected using a 1000 Hz spectral width. Unsuppressed spectra with 4 averages were acquired in the same voxel, using the same parameters except for a TR of 10 s to be used as an internal standard. Spectra were analyzed using the advanced magnetic resonance algorithm in the Java-based MR user interface software (jMRUI version 2.2 (13), as described earlier (12). Frequency estimates for myocardial and hepatic TG content were described with the assumption of Gaussian line shapes at 0.9, 1.3, and 2.1 parts per million (ppm), and for the unsuppressed spectra a Lorentzian fitting algorithm was used at 4.7 ppm. Lipid resonances of myocardial and hepatic TG at 1.3 ppm and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (TG/water × 100).

Left ventricular function

Imaging was performed using a body coil for radiofrequency transmission and a 5-element synergy coil for signal receiving. To assess systolic function, the heart was imaged in the short axis orientation using an ECG triggered, sensitivity encoding balanced steady state free procession sequence with breath-holds. Imaging parameters were: field-of-view = 400 × 320 mm, reconstructed matrix size = 256 × 256, slice thickness = 10 mm, slice gap = 0 mm, flip angle = 35°, TE = 1.67 ms, TR = 3.34 ms and 12 to 14 slices (dependent on the heart size). Temporal resolution was 25 to 39 ms, depending on the heart rate. Dedicated post processing software (MASS®, Medis, Leiden, the Netherlands) was used to assess left ventricular (LV) ejection fraction (EF) as described previously (14). To asses LV diastolic function, an ECG-gated, free-breathing gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve (15,16). Imaging parameters were: TE = 5 ms, TR = 14 ms, flip-angle = 20°, slice thickness = 8 mm, field of view = 350 mm, matrix size = 256 × 256, velocity encoding = 100 cm/s and scan percentage = 80%. Flow velocities in early diastole (E) and at atrial contraction (A) were measured and their peak flow ratio was calculated (E/A ratio) using FLOW® (Medis, Leiden, the Netherlands). Furthermore, we calculated the deceleration of the early filling phase (E deceleration). During MR imaging, blood pressure and heart rate were measured with an automatic device (Dinamap DPC100X, Freiburg, Germany).

Assays

Plasma glucose concentrations were measured by a continuous glucose monitoring system (Metronic MiniMed Inc., Northridge, CA, USA) and/ or (when not applicable) by the patients own device (at least each 2 hours during daytime and at 4-hour intervals during night time). Plasma levels of total cholesterol (TC) and TG concentrations were measured on a fully automated P800 analyzer (Roche, Almere, the Netherlands). Plasma NEFA levels were measured by using a commercial kit (NEFA-C; Wako Chemicals, Neuss, Germany). HbA1c levels were measured with an HPLC system (Variant, Biomed, Hercules, CA, USA).

Statistical Analysis

Statistical comparisons were performed with SPSS, version 14.0 (SPSS Inc., Chicago, IL, USA). Baseline measurements and measurements during partial insulin deprivation were compared by paired t-test. Data are shown as mean ± SEM. $P<0.05$ was considered to reflect significant differences.

RESULTS

Metabolic effects

Patient characteristics at baseline and during hyperglycemia are shown in Table 1. During partial insulin deprivation, hyperglycemic dysregulation was present in all patients (mean plasma 24-hour glucose was 8.4 ± 0.6 mmol/L during the control study which increased to 15.9 ± 0.8 mmol/L during partial insulin deprivation ($P<0.001$). Concomitantly, plasma NEFA levels increased from 0.31 ± 0.05 to 0.46 ± 0.07 mmol/L ($P=0.02$).

Myocardial and hepatic triglyceride content

Myocardial TG content was 0.31 ± 0.04 % at baseline and did not change during hyperglycemic dysregulation (0.34 ± 0.06 %, $P=0.6$). Hepatic TG content did not change either during hyperglycemic dysregulation (0.77 ± 0.09 % at baseline *vs*. 0.84 ± 0.11 % under hyperglycemic conditions, $P=0.4$).

Left ventricular function

Systolic and diastolic blood pressures and LVEF were unchanged during hyperglycemic dysregulation (Table 1). Furthermore, E deceleration did not change (4.4 $mL/s^2 \times 10^{-3}$ at baseline *vs*. 4.5 $mL/s^2 \times 10^{-3}$ during hyperglycemic conditions, $P=0.8$). E/A ratio was also unaffected (1.9 ± 0.2 at baseline *vs*. 1.9 ± 0.3 during hyperglycemic dysregulation, $P=0.9$).

Table 1. Clinical, MR spectroscopy and MR imaging parameters at baseline and during partial insulin deprivation

	Baseline	Partial insulin deprivation	P
Clinical characteristics			
Age (years)	41 ± 3		
HbA1c (%)	7.4 ± 0.2		
Duration of diabetes (years)	21.7 ± 2.3		
BMI (kg/m^2)	23.5 ± 0.6	23.0 ± 0.8	0.1
Mean 24h glucose (mmol/L)	8.4 ± 0.6	15.9 ± 0.8	< 0.001
Mean 24h insulin (U/L)	45 ± 5	27 ± 5	< 0.001
NEFA (mmol/L)	0.31 ± 0.05	0.46 ± 0.07	0.02
TG (mmol/L)	1.03 ± 0.24	0.85 ± 0.49	0.2
Total cholesterol (mmol/L)	4.3 ± 0.2	4.4 ± 0.2	0.1
Systolic blood pressure (mmHg)	114 ± 4	118 ± 5	0.2
Diastolic blood pressure (mmHg)	68 ± 3	73 ± 3	0.1
Heart rate (bpm)	63 ± 1	60 ± 3	0.3
MR spectroscopy			
Hepatic TG content (%)	0.77 ± 0.09	0.84 ± 0.11	0.4
Myocardial TG content (%)	0.31 ± 0.04	0.34 ± 0.06	0.6
MR imaging			
LV ejection fraction (%)	58 ± 1	59 ± 2	0.6
E peak filling rate (mL/s)	475 ± 21	467 ± 20	0.6
E deceleration (mL/s^2·10^{-3})	4.4 ± 0.4	4.5 ± 0.3	0.8
A peak filling rate (mL/s)	267 ± 21	254 ± 38	0.7
E/A peak ratio	1.9 ± 0.2	1.9 ± 0.3	0.9

Data are mean ± SEM. HbA1c, glycated haemoglobin; NEFA, non-esterified fatty acid; TG, triglyceride; LV, left ventricular, E, early filling phase; A, atrial filling phase

DISCUSSION

This study documents the effects of short-term hyperglycemic dysregulation on myocardial TG content and LV function in patients with type 1 diabetes. The present study shows that hyperglycemic dysregulation for 24 hours, as frequently observed in patients with T1DM, does not modulate myocardial TG content or myocardial function, despite considerable metabolic dysregulation.

We hypothesized that short-term partial insulin deprivation results in changes in myocardial TG content, possibly associated with changes in myocardial function. Stiffness of intermediate sized arteries is rapidly increased in patients with T1DM during hyperglycemia, whereas larger arteries seem unaffected (17). In healthy subjects myocardial function and TG content rapidly adapt to changes in metabolic state (5). Interestingly, this adaptation could

not be evoked by short-term hyperglycemic dysregulation, suggesting that the heart is protected from these short-term effects. Nonetheless, we can not exclude the possibility that prolongation of the duration of partial insulin deprivation might have resulted in changes in myocardial function and TG content.

Patients with T1DM have considerably altered myocardial glucose and fatty acid metabolism. Myocardial fatty acid utilization is increased whereas myocardial glucose uptake is considerably lower in diabetic patients compared with that in control subjects (2,4). These adaptations protect the heart to substrate overflow of the myocardium. Accordingly, the present study interestingly shows that myocardial TG content was not different in diabetic patients from the values we observed in studies in healthy subjects (5,12). However, fatty acid kinetics during hyperglycemia can not be derived from the present data and we can not exclude changes in myocardial fatty acid oxidation.

Hepatic TG content was measured to study the effects of insulin deprivation on tissue specific TG distribution, because we previously found discrepant effects of interventions on heart and liver (5). However, in the present study design insulin deprivation did not result in altered hepatic TG content.

In conclusion, short-term partial insulin deprivation resulting in hyperglycemic dysregulation, which is frequently observed in patients with type 1 diabetes, does not modulate myocardial TG content or LV function, despite considerable metabolic dysregulation.

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