Chapter 8

Short-term Hyperglycemic Dysregulation in Patients with Type 1 Diabetes Mellitus Does Not Change Myocardial Triglyceride Content or Myocardial Function

Adapted from Diabetes Care 2008; 31(8):1613-1614

S. Hammer
J.T. Jonker
H.J. Lamb
R.W. van der Meer
W. Zondag
J.M. Sepers
A. de Roos
J.W.A. Smit
J.A. Romijn
Chapter 8

116

SUMMARY

Objectives: Patients with type 1 diabetes mellitus (DM1) suffer from frequent episodes of hyperglycemia and high plasma levels of non-esterified fatty acids (NEFAs) despite insulin treatment. The aim of this study was to evaluate the effects of partial insulin deprivation with resulting hyperglycemia on myocardial triglyceride (TG) content and myocardial function in patients with DM1.

Materials and methods: Myocardial TG content and left ventricular (LV) systolic and diastolic function were measured in 10 patients with DM1 (mean ± standard error glycated hemoglobin: 7.4 ± 0.2%) using 1H magnetic resonance (MR) spectroscopy and MR imaging respectively during optimal glucoregulation 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 per 24 hours at baseline, whereas it was 27 ± 5 units per 24 hours during hyperglycemic conditions (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). This hyperglycemic dysregulation had no effects on myocardial TG content (0.31 ± 0.04 and 0.34 ± 0.06%, respectively, P = 0.587) and LV systolic or diastolic function.

Conclusions: Short-term hyperglycemic dysregulation, frequently observed in patients with DM1, does not modulate myocardial TG content or myocardial function, despite considerable metabolic adaptations. Apparently, the heart is protected from short-term metabolic effects of hyperglycemic dysregulation in patients with DM1 with respect to myocardial TG content and myocardial function.
INTRODUCTION

Intensive insulin treatment is the cornerstone of therapy of patients with type 1 diabetes mellitus (DM1). Nonetheless, this treatment is not fully able to restore glucoregulation to normal, and patients with DM1 suffer from frequent episodes of less optimal metabolic regulation, reflected in short- or longer lasting episodes of hyperglycemia. These problems in optimization of glucoregulation may also result in altered lipid metabolism. For instance, an increased amount of intramyocellular lipid content was observed in the soleus and tibialis anterior muscles in patients with DM1 compared to controls (1). In these patients with DM1 there was an association between intramyocellular lipid content and the degree of glucoregulation reflected by glycated hemoglobin (HbA1c) values (1). These findings suggest a major role of metabolic dysregulation in the induction of abnormal intramyocellular lipid accumulation in DM1.

The metabolic effects of DM1 also extend to the heart. Studies with positron emission tomography have documented that patients with DM1 exhibit increased myocardial fatty acid utilization and oxidation, 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). The authors studied patients with DM1 during euglycemia, hyperlipidemia, and a hyperinsulinemic-euglycemic clamp and concluded that insulin and plasma NEFA levels can regulate the intramyocardial fate of fatty acids in humans with DM1. Therefore, it is likely that cardiac metabolism is also affected by episodes of metabolic dysregulation in patients with DM1.

In several conditions there is a discrepancy between fatty acid uptake and fatty acid utilization in the heart, reflected in alterations in myocardial triglyceride (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 myocardial TG accumulation 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 DM1 due to insufficient insulin provision may also modulate myocardial TG content and possibly 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 DM1, otherwise well-controlled by continuous insulin pumps. For this purpose, the subjects were studied twice, during intensive insulin treatment and, on a separate occasion, after 24 hours of a ~50% reduction in baseline insulin infusions, resulting in hyperglycemia. Myocardial TG content and myocardial function were measured using hydrogen 1 magnetic resonance spectroscopy (1HMRS) and magnetic resonance (MR) imaging,
respectively. To assess the tissue-specificity of the potential changes in myocardial TG content, we also measured hepatic TG content on the two occasions with $^1$HMRS.

**MATERIALS AND METHODS**

**Patients**

We studied 10 (mean age ± standard error: 41 ± 11 years), C-peptide negative patients with DM1 (5 men, HbA1c 7.4 ± 0.2%). 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 (5). The mean duration of DM1 was 21.7 ± 2.3 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, active retinopathy, the 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 (body mass index > 30 kg/m²) 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 period, in which subjects aimed at optimal blood glucose levels by intensive insulin treatment by insulin pump therapy, combined with frequent assessments of blood glucose levels. The subjects documented their (normal) caloric intake and were restricted from alcohol. Patients were also instructed to document their basal and bolus insulin infusions. The second occasion was performed 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. The 3 days prior to evaluation, patients were again instructed to maintain the same caloric intake as for baseline measurements.

Blood glucose levels were monitored from 3 days prior to baseline and hyperglycemic measurements with a continuous glucose monitoring system (Metronic MiniMed Inc., Northridge, CA, USA). Furthermore, patients self-measured and documented their plasma glucose levels and ensured blood glucose levels remained < 20 mmol/l during targeted partial insulin deprivation. When blood glucose levels raised above 20 mmol/l patients were instructed to infuse insulin to maintain levels between 15 mmol/l and 20 mmol/l. At each study occasion,
post absorptive blood samples were obtained and we performed $^{1}$HMRS and MR imaging. The sequence between the 2 occasions was assigned by balanced assignment.

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

All MR measurements were performed on a 1.5-Tesla Gyroscan ACS-NT MR imaging scanner (Philips Medical Systems, Best, The Netherlands) with patients in the supine position at rest. MR data was obtained in the afternoon. For $^{1}$HMRS 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 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 trigger 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 10000 ms 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). Peak estimates of lipid resonances of myocardial and hepatic TGs at 1.3 parts per million (ppm) and 0.9 ppm were summed and calculated as a percentage of the unsuppressed water signal (TGs/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 = 1.7 ms, TR = 3.4 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 assess 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 = 4.8 ms, TR = 14 ms, flip angle = 20°, slice thickness = 8 mm, field of view = 350 mm$^2$, 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 aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), γ-glutamyl transferase (γGT), 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, Ill, USA). Baseline measurements and measurements during partial insulin deprivation were compared by paired t-tests. Data are shown as mean ± standard error. \( P < 0.05 \) was considered to reflect significant differences.

RESULTS

Metabolic effects
Patient characteristics at baseline and during hyperglycemia are shown in Table 8.1. Mean insulin infusion rate was 45 ± 5 units per 24 hours during the control study, whereas it was only 27 ± 5 units per 24 hours during partial insulin deprivation \( (P < 0.001) \). 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 \( (P = 0.015) \). Furthermore, plasma AP concentrations increased from 79 ± 6 to 88 ± 4 U/l \( (P = 0.008) \) and plasma AST concentrations decreased from 37 ± 4 to 28 ± 3 U/l \( (P = 0.004) \).

Myocardial and hepatic triglyceride content
Myocardial TG content was 0.31 ± 0.04% at baseline and did not change during hyperglycemic dysregulation \( (0.34 ± 0.06%, P = 0.587) \). In addition, hepatic TG content did not change during
Myocardial Effects of Hyperglycemia

Hyperglycemic dysregulation (0.77 ± 0.09% at baseline vs 0.84 ± 0.11% under hyperglycemic conditions, \( P = 0.400 \)).

Left ventricular function

Systolic and diastolic blood pressures and LVEF were unchanged during hyperglycemic dysregulation (Table 8.2). Furthermore, E deceleration did not change (4.4 ml/s² × 10⁻³ at baseline vs 4.5 ml/s² × 10⁻³ during hyperglycemic conditions \( P = 0.777 \)). E/A ratio was also unaffected (1.9 ± 0.2 at baseline vs 1.9 ± 0.3 during hyperglycemic dysregulation, \( P = 0.854 \)).

Table 8.1. Metabolic parameters at baseline and after insulin reduction.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>41 ± 3</td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.5 ± 0.6</td>
<td>23.0 ± 0.8</td>
</tr>
<tr>
<td>Plasma mean 24 hour glucose (mmol/l)</td>
<td>8.4 ± 0.6</td>
<td>15.9 ± 0.8*</td>
</tr>
<tr>
<td>Plasma mean 24 hour insulin (U/l)</td>
<td>45 ± 5</td>
<td>27 ± 5*</td>
</tr>
<tr>
<td>Plasma AP (mmol/l)</td>
<td>79 ± 6</td>
<td>88 ± 4†</td>
</tr>
<tr>
<td>Plasma AST (mmol/l)</td>
<td>37 ± 4</td>
<td>28 ± 3†</td>
</tr>
<tr>
<td>Plasma ALT (mmol/l)</td>
<td>24 ± 3</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Plasma yGT (mmol/l)</td>
<td>21 ± 2</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/l)</td>
<td>4.3 ± 0.2</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Plasma non-esterified fatty acids (mmol/l)</td>
<td>0.31 ± 0.05</td>
<td>0.46 ± 0.07†</td>
</tr>
<tr>
<td>Plasma TGs (mmol/l)</td>
<td>1.03 ± 0.24</td>
<td>0.85 ± 0.49</td>
</tr>
<tr>
<td>Liver TG content (%)</td>
<td>0.77 ± 0.09</td>
<td>0.84 ± 0.11</td>
</tr>
<tr>
<td>Myocardial TG content (%)</td>
<td>0.31 ± 0.04</td>
<td>0.34 ± 0.06</td>
</tr>
</tbody>
</table>

\( P < 0.001, † P < 0.05 \) vs baseline. Data are mean ± standard error.

HbA1c = glycated hemoglobin, AP = alkaline phosphatase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, γGT = gamma-glutanyl transferase, TG = triglyceride.

Table 8.2. Parameters of myocardial function at baseline and during hyperglycemia.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>114 ± 4</td>
<td>118 ± 5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68 ± 3</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>63 ± 1</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>LV Ejection fraction (%)</td>
<td>58 ± 1</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>E peak filling rate (ml/s)</td>
<td>475 ± 21</td>
<td>467 ± 20</td>
</tr>
<tr>
<td>E deceleration (ml/s² × 10⁻³)</td>
<td>4.4 ± 0.4</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>A peak filling rate (ml/s)</td>
<td>267 ± 21</td>
<td>254 ± 38</td>
</tr>
<tr>
<td>E/A peak ratio</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.3</td>
</tr>
</tbody>
</table>

Data are mean ± standard error.

LV = left ventricular, E = early filling phase, A = atrial filling phase.
DISCUSSION

The present study was designed to study clinically relevant episodes of hyperglycemic dysregulation, frequently observed in patients with DM1. The study shows that hyperglycemic dysregulation for 24 hours does not influence myocardial TG content or myocardial function, despite considerable metabolic alterations. Moreover, hepatic TG content was not affected by short-term partial insulin deprivation in patients with DM1.

Hyperglycemic dysregulation did not alter LV (diastolic) heart function in the present study. Others could not document conclusive effects of hyperglycemia on myocardial blood flow (17) and vascular function (18). To our knowledge, this is the first study to document the effects of short-term hyperglycemic dysregulation on LV myocardial function in humans in vivo. Although our results suggest that short-term hyperglycemia does not alter myocardial function or myocardial TG content, we can not exclude the possibility that prolongation of the duration of partial insulin deprivation beyond 24 hours might have resulted in changes in cardiac function and myocardial TG accumulation. Nonetheless, the present study reflects a realistic clinical situation, as short-term hyperglycemic dysregulation frequently occurs in patients with DM1.

The present study was initiated, since we anticipated that partial insulin deprivation would also result in changes in myocardial TG content, possibly associated with changes in myocardial function. Targeted hypoinsulinemia in patients with DM1 indeed increased plasma levels of NEFAs by increasing adipose tissue lipolysis, resembling the effects of caloric restriction on plasma NEFA levels in healthy subjects (5;6). During caloric restriction, the resulting increased availability of plasma NEFAs considerably exceeds the oxidative requirements of fatty acids (19). In accordance with those observations, caloric restriction induces myocardial TG accumulation in healthy subjects (5;6;20). Moreover, in obesity and type 2 diabetes mellitus chronically elevated plasma NEFA levels are associated with increased myocardial TG content (21). These increased TG stores are associated with impaired myocardial function (22;23). In the present study in patients with DM1, insulin deficiency was introduced by targeted reduction of intensive insulin therapy. This resulted in considerable hyperglycemia and increased NEFA levels, but this excess of plasma energy substrates apparently did not result in myocardial TG accumulation.

Patients with DM1 have considerably altered myocardial glucose and fatty acid metabolism. Myocardial fatty acid utilization is increased in patients with DM1 compared to healthy subjects (2). Moreover, a larger proportion of myocardial fatty acid utilization is oxidized in patients with DM1, whereas myocardial glucose uptake is considerably lower in patients compared to controls (2;4). These changes protect the heart to substrate overflow of the myocardium. Accordingly, in the present study, myocardial TG content was not different in patients with DM1 from the values we observed in previous studies in healthy subjects (5;6;12). However, in healthy subjects myocardial function and TG content rapidly adapt to changes in nutritional intake, associated with considerable changes in plasma levels of NEFAs.
Hepatic TG content was measured to study the potential organ differential distribution of fatty acids between the heart and the liver as has been observed before (5;6). Baseline values of hepatic TG content were in the normal range in this particular cohort of patients, probably due to the intensive insulin treatment in these subjects, also reflected in relatively low HbA1c levels. Nonetheless, partial insulin deficiency resulted in changes in hepatic functions although dissociated from the unchanged hepatic TG content. We observed an increase in plasma AP levels and a decrease in AST levels during hyperglycemic dysregulation. Apparently, in this study, hepatic TG content is not a good parameter reflecting changes in hepatic metabolism, other than reflecting the net balance between fatty acid uptake, de novo lipogenesis, fatty acid oxidation and very low-density lipoprotein TG secretion.

CONCLUSIONS

In conclusion, the present study shows that short-term hyperglycemic dysregulation, which is frequently observed in patients with DM1, does not alter myocardial TG content or LV function, despite considerable metabolic adaptations. The study for the first time documents the myocardial effects of hyperglycemic dysregulation in patients with DM1. Apparently, the heart is protected from short-term metabolic effects of hyperglycemic dysregulation in patients with DM1 with respect to myocardial TG content and myocardial function.

ACKNOWLEDGMENTS

We gratefully thank Marja Dijk-Schaap and Nathalie Masurel for their assistance with the study.
REFERENCES


