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Ectopic fat depositions in obesity and diabetes : nutritional, exercise and pharmacological interventions

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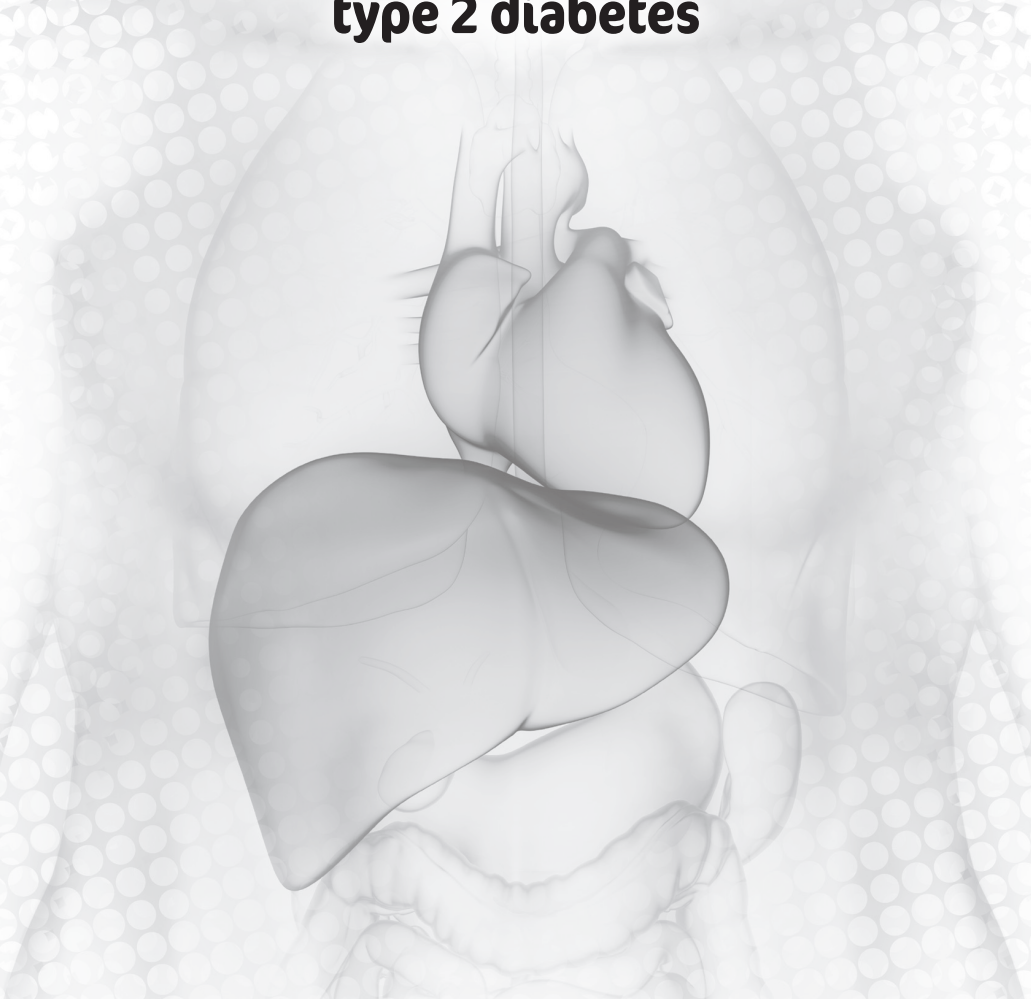
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Chapter 3

Effects of hepatic triglyceride content on myocardial metabolism in type 2 diabetes

An anatomical illustration of the human torso, showing the heart and liver. The heart is positioned in the upper chest area, and the liver is located in the upper abdominal area. The illustration is rendered in a light, semi-transparent style, allowing the underlying structures to be visible. The background of the entire page features a pattern of small, light-colored circles.

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ABSTRACT

Background

Heart disease is the leading cause of mortality in type 2 diabetes (T2DM). Central obesity and hepatic steatosis, both hallmark abnormalities in T2DM, have been related to increased risk of heart disease.

The purpose of this study was to investigate the relationship between hepatic triglyceride content and both myocardial function and metabolism in T2DM.

Methods

Sixty-one T2DM patients underwent myocardial perfusion and substrate metabolism measurements by positron-emission tomography, using ^{15}O -water, ^{11}C -palmitate and ^{18}F -2-fluoro-2-deoxy-D-glucose. In addition, whole-body insulin sensitivity (M/I) was determined. Myocardial left ventricular (LV) function and high-energy phosphate metabolism were measured using magnetic resonance (MR) imaging and ^{31}P -MR spectroscopy respectively. Hepatic triglyceride content was measured by ^1H -MR spectroscopy. Patients were divided according to hepatic triglyceride content (T2DM-low $\leq 5.56\%$ versus T2DM-high $> 5.56\%$).

Results

In addition to decreased M/I ($P=0.002$), T2DM-high patients had reduced myocardial perfusion ($P=0.001$), glucose uptake ($P=0.005$) and PCr/ATP ratio ($P=0.003$), compared with T2DM-low patients whereas cardiac fatty-acid metabolism and LV function were not different. Hepatic triglyceride content correlated inversely with M/I (Pearsons' $r=-0.620$, $P<0.001$), myocardial glucose uptake ($r=-0.413$, $P=0.001$) and PCr/ATP ($r=-0.442$, $P=0.027$). Insulin sensitivity correlated positively with myocardial glucose uptake ($r=0.528$, $P<0.001$) and borderline with myocardial PCr/ATP ($r=0.367$, $P=0.072$), whilst a positive association was found between cardiac glucose uptake and PCr/ATP ($r=0.481$, $P=0.015$).

Conclusion

High liver triglyceride content in T2DM was associated with decreased myocardial perfusion, glucose uptake and high-energy phosphate metabolism, in conjunction with impaired whole-body insulin sensitivity. The long-term clinical implications of hepatic steatosis with respect to cardiac metabolism and function in the course of T2DM require further study.

INTRODUCTION

In the past few decades, the prevalence of obesity and type 2 diabetes (T2DM) has grown to epidemic proportions (1). T2DM patients are at increased risk of cardiovascular disease (CVD), in particular coronary artery disease (CAD) and heart failure, and consequently, heart disease is the most common cause of death in T2DM (2,3). Cardiac abnormalities in T2DM patients can, however, develop in the absence of hypertension or coronary artery disease. These myocardial derangements are attributed to diabetic cardiomyopathy (DCM) (4), a disease entity with a high propensity to progress into overt congestive heart failure (5).

Several mechanisms have been proposed to underlie DCM(4), in particular the metabolic hallmarks of the T2DM phenotype such as insulin resistance, dyslipidemia and hyperglycemia. These diabetes-related metabolic derangements are collectively thought to contribute to altered myocardial substrate handling and, subsequently, to the observed cardiac (diastolic) dysfunction (4). The underlying mechanisms include oxidative stress, mitochondrial dysfunction and compromised high-energy phosphate metabolism (4).

Hepatic steatosis is a common finding in patients with (uncomplicated) T2DM. It is associated with a cluster of metabolic abnormalities, including insulin resistance, hyperglycemia, dyslipidemia and a pro-inflammatory state; all factors known to adversely affect the cardiovascular system (6,7). Indeed, several studies in humans showed an association of fatty liver with an increased carotid intima media thickness (cIMT) (8), impaired flow-mediated vasodilation (9), advanced atherosclerosis (10), and CVD / CAD(6). Interestingly, high liver fat was associated with alterations in myocardial high-energy phosphate metabolism in healthy obese individuals (11) and myocardial glucose metabolism in T2DM patients with CAD (12). These studies have provided hypothesis generating preliminary data regarding the potential mechanisms that could link liver steatosis to cardiac disease as a risk factor for CVD. However, whether high liver fat adversely affects metabolic or functional aspects of the heart in asymptomatic patients with uncomplicated T2DM, still remains to be determined.

Using state-of-the-art imaging techniques, the aim of the present study was to assess the relationship between liver triglyceride content and myocardial metabolism in T2DM patients with verified absence of clinical ischemic heart disease.

METHODS

Participants

Sixty-one T2DM patients were selected from a previous study based on availability of PET measurements (13). This two-center study was approved by the Medical Ethics Committee of both centers, and performed in compliance with the Declaration of Helsinki. All patients signed informed consent prior to inclusion. Patients were recruited by advertisements in

local newspapers. Male T2DM patients, aged 45-65 years, without diabetes related complications were eligible. Inclusion criteria were a glycated hemoglobin A_{1c} (HbA_{1c}) level of 6.5-8.5 % at screening, BMI of 25-32 kg/m², blood pressure not exceeding 150/85 mm Hg (with or without the use of anti-hypertensives). Patients were excluded if they had a history or current hepatic or cardiovascular disease and if they used insulin, fibrates, thiazolidinediones or other hormonal replacement therapy. Screening of patients consisted of medical history, physical examination, echocardiogram and fasting blood and urine analyses. In addition, patients underwent dobutamine-stress echocardiography to confirm absence of inducible ischemia. After successful screening, participants commenced with a 10-week run-in period during which their regular blood glucose lowering agents were changed to glimepiride monotherapy, and titrated until a stable dose was reached during 8 weeks prior to assessments, to exclude possible confounding effects on myocardial metabolism of differential agents. The present baseline data were derived from a previously reported intervention study (13), in which participants were randomized to pioglitazone or metformin after baseline measurements, to study the effects of these agents on cardiac function and metabolism.

Imaging

The study protocol was performed during two visits within the same week. During one visit, cardiac perfusion and substrate metabolism were measured using PET, whereas during the other visit myocardial function was measured by MRI and hepatic triglyceride content and myocardial PCr/ATP ratio by ¹H- and ³¹P-spectroscopy respectively. At both occasions, patients visited the clinical research unit at 08:00 in the morning following an overnight fast of approximately 12-15 hours. Glucose-lowering agents were not taken in the morning before assessments.

PET

All PET examinations were performed at one single center (Amsterdam) using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA). Patients received two catheters; one in an antecubital vein and one in a contralateral hand vein being wrapped into a heated blanket to obtain arterialized blood. Myocardial perfusion (MBF) studies were performed in 2D acquisition using ¹⁵O-water (1100 MBq) as tracer. Myocardial glucose metabolism (MMRglu) and fatty-acid uptake studies were performed in 3D acquisition mode using ¹⁸F -2-fluoro-2-deoxy-D-glucose (¹⁸F-FDG, 170 MBq) and ¹¹C-palmitate (185 MBq) as tracers, respectively. MBF and fatty-acid uptake were assessed in the fasting state, whereas MMRglu was measured under hyperinsulinemic euglycemic conditions. The scan protocol was as follows: following a 10-min transmission scan for attenuation correction, ¹⁵O-water was injected (t=10 min) and a 10-min dynamic emission scan consisting of 40 frames with progressively increasing frame length was acquired. Subsequently, a 30-min dynamic emission scan consisting of 34 frames with increasing frame length was performed following ¹¹C-palmitate injection (t=35 min).

Hereafter, the clamp was started ($t=65$ min), as described previously (14), to approximate an isometabolic steady state (plasma glucose level 5 mmol/L) and measure whole-body insulin sensitivity (M value). At steady state ($t=155$ min), following a new transmission scan, ^{18}F -FDG was injected and a 60-min dynamic emission with increasing frame length of 40 frames was acquired. Blood samples were collected during all three scans at predefined time points to measure glucose, NEFA, lactate, lipids and insulin levels. In addition, ^{11}C - CO_2 was measured during the ^{11}C -palmitate scan (15). Total radiation exposure of the entire sequence of scans was 4.87 mSv.

PET data analysis

PET data were reconstructed using filtered back projection applying all appropriate corrections. In order to generate myocardial time-activity curves, region of interests (ROIs) were defined on resliced LV short-axis (summed) ^{11}C -palmitate and ^{18}F -FDG images and subsequently projected onto the corresponding dynamic images. ROIs were drawn and grouped for further analysis as previously described (16). Myocardial segments exposed to liver spill-in were omitted from the analysis of ^{11}C -palmitate data. Additional ROIs were defined in right and left ventricular chambers for ^{11}C -palmitate and ^{15}O -water image-derived input functions (IDIF). A separate aorta ascendence ROI was defined for ^{18}F -FDG IDIF. Myocardial perfusion was determined using the standard single-tissue compartment model (17). As resting myocardial perfusion is related to the rate pressure product ($\text{RPP} = \text{heart rate} \times \text{systolic blood pressure}$), corrected resting MBF ($1000 \times \text{MBF}/\text{RPP}$) was also calculated (18). Moreover, myocardial vascular resistance was calculated by dividing the mean arterial pressure by myocardial rest perfusion (18). ^{11}C -palmitate time-activity curves were analyzed using a three-tissue plasma input kinetic model, which, together with plasma NEFA concentrations, enabled calculation of Myocardial fatty acid uptake, oxidation and esterification (MFAU, MFAO and MFAE, respectively) (13,19). The ^{11}C -palmitate IDIF was corrected for ^{11}C - CO_2 metabolites and differences between plasma and whole blood concentrations as described elsewhere (13,19). MMRglu was calculated by multiplying the net influx constant for ^{18}F -FDG, K_i , with the mean plasma glucose concentration. For determination of K_i , Patlak graphical analysis was used (20).

MR imaging

The heart was imaged in short-axis orientation using electrocardiographically (ECG)-gated breath-holds with a sensitivity encoding balanced turbo-field echo sequence. LV ejection fraction (LVEF), cardiac output (CO), stroke volume (SV), LV end-diastolic (LVEDV) and LV end-systolic volume (LVESV), LV mass and their indexes calculated by dividing each parameter through the body surface area (CI, LVEDVI, LVESVI and LVMI), were determined by analyses of end-diastolic and end-systolic images (21). An ECG-gated gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve in order to determine LV diastolic function. Measures included the peak filling rate of the early filling

phase (E) and of the atrial contraction (A), the ratio of the peak filling rates (E/A) and the peak deceleration gradient of the early filling phase (E deceleration peak). Additionally, an estimation of LV filling pressure (E/Ea) was calculated (22). During MR imaging, blood pressure and heart rate were measured.

To quantify the amount of visceral and subcutaneous abdominal fat, three consecutive transverse images were obtained during 1 breath hold with the middle image at a level just above the fifth lumbar vertebra (23). All images were analyzed quantitatively using dedicated software (FLOW or MASS, Medis, Leiden, the Netherlands).

Hepatic ^1H -MRS

Hepatic ^1H -MR spectroscopy was performed as described previously (21,24). Briefly, to obtain hepatic ^1H -MR spectra, an 8 ml voxel was positioned in the liver avoiding gross vascular structures and adipose tissue depots. Both spectra with and without water suppression were obtained, to calculate hepatic triglyceride (TG) content as a percentage relative to water ($100 \times \text{TG}/\text{water}$) (24). To quantify hepatic triglyceride content, the methylene and water signals were corrected for T_2 decay by use of the exponential relaxation equation $S_c = S_0 \cdot \exp(-TE/T_2)$, where S_c represents the corrected signal, TE the echo time, T_2 the longitudinal relaxation time and S_0 the signal after application of the 90° pulse. T_2 relaxation times for water and triglyceride of 50 and 60 ms, respectively, were used as described by Szczepaniak et al. (24). A fatty liver was defined as hepatic triglyceride content exceeding 5.56% (24).

Myocardial ^{31}P -MRS

ECG-triggered ^{31}P -MR spectra of the LV anterior wall were acquired using a 100-mm-diameter surface coil. Volumes of interest were selected by image-guided spectroscopy with 3D-ISIS. Shimming was performed automatically and tuning and matching of the ^{31}P -surface coil was performed manually. Spectroscopic volume size typically was $7 \times 7 \times 7$ cm. Acquisitions were based on 192 averaged free induction decays, and total acquisition time was 10 min. ^{31}P -MR spectra were corrected for partial saturation effects and for the adenosine-triphosphate (ATP) contribution from blood in the cardiac chambers. The myocardial phosphocreatine (PCr)/ATP ratios of the spectra were calculated and used as a parameter representing myocardial HEP metabolism (25,26).

Biochemical analyses

Samples were analyzed at one certified central laboratory (Amsterdam, The Netherlands). HbA_{1c} was determined by high-performance liquid chromatography (HPLC; Menarini Diagnostics, Florence, Italy; reference values: 4.3-6.1%). Plasma glucose was quantified by the use of a hexokinase-based technique (Roche Diagnostics, Mannheim, Germany). Plasma triglycerides, total cholesterol and high-density lipoprotein cholesterol were determined using enzymatic colorimetric methods (Modular, Hitachi, Japan). Levels of low-density lipoprotein

cholesterol were calculated using Friedewald's formula (reference values: 2.0-4.6 mmol/L). Plasma insulin levels were quantified by an immunoradiometric assay (Bayer Diagnostics, Mijdrecht, The Netherlands). Plasma free-fatty acids were measured by an enzyme-linked immunoabsorbent assay (Wako Chemicals, Neuss, Germany). Ultra-sensitive C-reactive protein (us-CRP) was determined by ELISA (DSL, Webster, Texas, USA). The sensitivity was 1.6 µg/L and the interassay coefficients of variations ranged from 3 to 5%. In duplo determinations of plasma malondialdehyde, a marker of oxidative stress, were performed by HPLC after alkaline hydrolysis and reaction with thiobarbituric acid (27). The intra-assay COV was 5.7%.

Statistical analysis

Values are expressed as mean $\pm$ (standard error; SEM) or median (with interquartile range; IQR). Normality was assumed if the histogram showed a normal distribution, the Kolmogorov-Smirnoff test was > 0.05 and skewness and kurtosis were < 1.0 . Non-normally distributed data were log-transformed. Comparisons between groups were made using the independent t-tests. Linear regression was used to adjust for BMI differences between groups. The chi-square test was used for nominal parameters. Univariate and multiple analyses with a forward selection procedure were performed. The aim of these analyses was to determine which variables influence liver triglyceride content, MMRglu, PCr/ATP. Those variables with a $P < 0.1$ were subsequently entered in a forward multivariable regression analysis and those variables with a $P < 0.05$ were considered independently related to the dependent variable. As liver triglyceride content was a skewed variable, this variable was log-transformed. Moreover, several independent variables were log-transformed. Analyses were performed with SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). A two-tailed probability value < 0.05 was considered significant.

RESULTS

Assessment of myocardial function, hepatic ^1H -MR spectroscopy and measurements of MMRglu were successfully completed in all 61 patients. Because of technical reasons ^{15}O -water and ^{11}C -palmitate data were not available for 1 and 8 subjects, respectively. Due to the demanding nature of the protocol myocardial PCr/ATP was offered as an optional test. Therefore measurements were available only in a subgroup of 25 patients. These subjects did not differ in clinical characteristics from the other 36 patients.

Subject characteristics, hemodynamics and myocardial function

Table 1 shows characteristics of the entire study population, divided into groups with high and low hepatic triglyceride content. Both groups did not differ with respect to age, glycemic control, duration of diabetes and use of medication. BMI and plasma triglycerides,

however, were higher in the T2DM-high group. Metabolic characteristics are given in Table 2, showing higher fasting plasma insulin and lactate and a borderline significant increase in CRP in T2DM-high patients. Under hyperinsulinemic euglycemic clamp conditions, plasma fatty-acid and insulin levels were higher and M/I was lower in T2DM-high patients. No differences were observed in myocardial hemodynamics, and LV systolic and diastolic function and dimensions between T2DM-high and T2DM-low patients (Table 3).

Table 1. Subject characteristics

	T2DM-low N=29	T2DM-high N=32	P value
Age (yrs)	57.3 ± 0.9	56.4 ± 1.0	0.507
Time since diagnosis of diabetes (yrs)	4 (2-6)	4 (3-5)	0.858
Current smoker number (%)	21 (6/29)	22 (7/32)	0.230
Body mass index (kg/m ²)	27.1 ± 0.6	30.1 ± 0.6	0.001
Waist circumference (cm)	101 ± 2	107 ± 2	0.015
HbA1c (%)	7.0 ± 0.2	7.3 ± 0.2	0.306
Total cholesterol (mmol/L)	4.3 ± 0.1	4.5 ± 0.1	0.212
LDL cholesterol (mmol/L)	2.6 ± 0.1	2.7 ± 0.1	0.467
HDL cholesterol (mmol/L)	1.05 (0.85-1.23)	0.96 (0.81-1.10)	0.131
Triglycerides (mmol/L)	1.2 (0.8-1.6)	1.8 (1.2-2.4)	0.054
ALT (U/L)	26 (21-33)	36 (29-49)	0.004
γGT (U/L)	21 (18-37)	41 (33-48)	< 0.001
Medications (%)			
Statins	38 (11/29)	47 (15/32)	0.481
Any antihypertensive medication	38 (11/29)	44 (14/32)	0.644
β-blockers	10 (3/29)	9 (3/32)	0.899
Diuretics	7 (2/29)	16 (5/32)	0.285
ACE inhibitors	17 (5/29)	19 (6/32)	0.878
Angiotensin II blocker	14 (4/29)	13 (4/32)	0.881
Calcium antagonists	3 (1/29)	6 (2/32)	0.613
Omega-3	1 (0/29)	0 (0/32)	0.337

Data are mean ± SEM or median (interquartile range).

ACE, angiotensin-converting enzyme; ALT, alanine aminotransferase; γGT, gammaglutamyl transferase; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T2DM-high or -low, type 2 diabetes patients with high or low liver triglyceride content

Table 2. Biochemical and metabolic characteristics of the study population

	T2DM-low (n=29)	T2DM-high (n=32)	P value
Fasting			
Plasma glucose (mmol/L)	8.3 (6.8-10.7)	8.3 (6.9-9.6)	0.600
Plasma non-esterified fatty acids (nmol/L)	480 (365-685)	510 (425-595)	0.986
Plasma lactate (mmol/L)	1.0 (0.9-1.3)	1.2 (1.0-1.5)	0.030
Plasma insulin (pmol/L)	54 ± 7	90 ± 10	0.006
usCRP (mg/L)	3.6 ± 0.6	7.0 ± 1.4	0.064
Malondialdehyde (μmol/L)	9.9 ± 0.5	9.8 ± 0.4	0.976
During hyperinsulinemia			
Plasma non-esterified fatty acids (nmol/L)	71 ± 11	130 ± 13	0.001
Plasma lactate (mmol/L)	1.1 (0.9-1.3)	1.1 (1.0-1.3)	0.754
Plasma insulin (pmol/L)	535 (458-611)	615 (571-744)	0.001
M/I value ((mg/kg/min)/(pmol/L))	0.62 (0.44-1.07)	0.37 (0.17-0.46)	0.002

Data are mean ± SEM or median (interquartile range). T2DM-high or -low, type 2 diabetes patients with high or low liver triglyceride content; usCRP, ultrasensitive C-reactive protein; M/I value, whole body insulin sensitivity adjusted during the steady state

Table 3. Hemodynamic parameters, cardiac dimensions and function in the study population

	T2DM-low (n=29)	T2DM-high (n=32)	P value
Hemodynamics			
Systolic blood pressure (mmHg)	122 ± 2	125 ± 2	0.443
Diastolic blood pressure (mmHg)	74 ± 1	76 ± 1	0.646
Heart rate (bpm)	63 ± 2	62 ± 1	0.794
Rate pressure product (bpm·mmHg)	7743 ± 288	7811 ± 243	0.857
Systolic function and dimensions			
LV mass (g)	104 ± 3	111 ± 3	0.154
LV mass index (g/m ²)	51 ± 1	52 ± 1	0.519
LV end-systolic volume (mL)	62 ± 2	62 ± 2	0.974
LV end-systolic volume index (mL/m ²)	30 ± 1	29 ± 1	0.439
LV stroke volume (mL)	93 ± 3	94 ± 3	0.630
Ejection fraction (%)	60 ± 1	60 ± 1	0.717
Cardiac index (L/min/m ²)	3.0 ± 0.1	2.9 ± 0.1	0.512
Cardiac work (mmHg/mL/min)	52.6 ± 0.2	53.7 ± 0.2	0.677

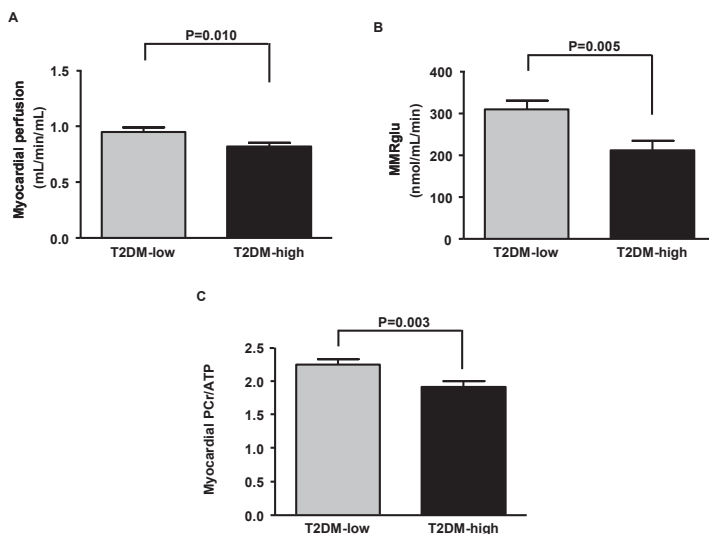
Table 3. (continued)

	T2DM-low (n=29)	T2DM-high (n=32)	P value
Diastolic function and dimensions			
LV end-diastolic volume (mL)	155 ± 4	157 ± 4	0.719
LV end-diastolic volume index (mL/m ²)	76 ± 2	73 ± 2	0.359
E peak filling rate (mL/s)	409 ± 15	413 ± 16	0.829
E deceleration peak (mL/s ² ·10 ⁻³)	3.37 ± 0.19	3.59 ± 0.19	0.459
E/A ratio	1.05 ± 0.05	1.03 ± 0.04	0.698
E/Ea	9.8 (7.7-13.0)	8.5 (6.6-11.4)	0.196

Data are mean ± SEM or median (interquartile range). T2DM-high or -low, type 2 diabetes patients with high or low liver triglyceride content; bpm, beats per minute; LV, left ventricular; E, early diastolic filling phase; A, diastolic atrial contraction; E/Ea, estimate of the left ventricular filling pressure

Myocardial perfusion and metabolism

MBF was lower in T2DM-high than in T2DM-low patients (Figure 1A), also after correction for RPPP (1.07 ± 0.04 versus 1.26 ± 0.05 mL·g⁻¹·mmHg⁻¹·10.000⁻¹, $P=0.006$). In contrast, myocardial vascular resistance was higher (118 ± 5 versus 98 ± 4 Hg·mL⁻¹·min⁻¹·mL⁻¹, $P=0.004$). Both MMRglu (Figure 1B) and PCr/ATP ratio (Figure 1C) were lower in T2DM-high than in T2DM-low patients. The alterations in myocardial fatty-acid metabolism in T2DM-high patients did not reach statistical significance (MFAU: 83 ± 5 versus 92 ± 7 nmol·min⁻¹·mL⁻¹, $P=0.266$; MFAO: 82 ± 5 versus 89 ± 6 nmol·min⁻¹·mL⁻¹, $P=0.361$; MFAE: 1 ± 1 versus 3 ± 1 nmol·min⁻¹·mL⁻¹, $P=0.368$).

Figure 1. Myocardial perfusion, glucose uptake and high-energy phosphate metabolism

Myocardial resting perfusion (A, n=61), Metabolic rate of glucose metabolism (MMRglu) (B, n=61), and myocardial phosphocreatine (PCr)/ATP ratio (C, n=25) in T2DM patients with low (gray bars) and high (black bars) liver triglyceride content

Table 4. Univariable and multivariable linear regression analysis of liver triglyceride content, myocardial metabolic rate of glucose and PCr/ATP ratio

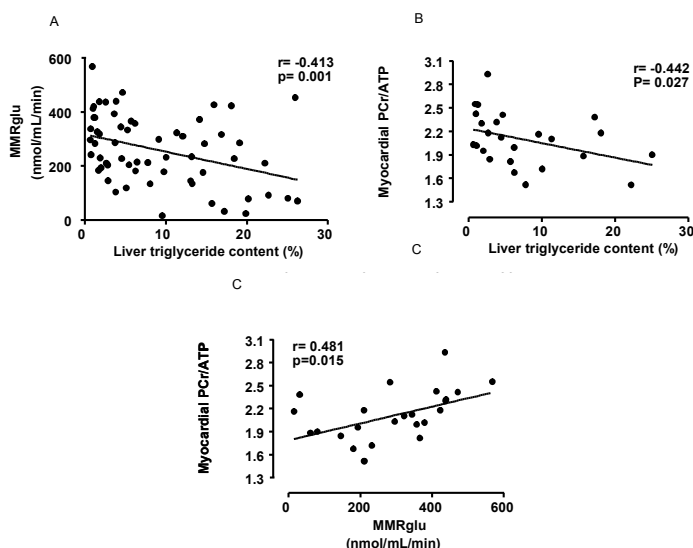
	Liver Triglyceride Content				MMRglu-10				PCr/ATP			
	Univariable		Multivariable		Univariable		Multivariable		Univariable		Multivariable	
	Retransformed regression coefficient	P value	Retransformed regression coefficient	P value	Regression coefficient	P value	Regression coefficient	P value	Regression coefficient	P value	Regression coefficient	P value
Plasma triglycerides (mmol/L)*	4.943	0.004	-	-	0.033	0.025	-	-	0.600	0.386	-	-
Plasma lactate (mmol/L)*	19.953	0.004	-	-	0.001	0.001	0.001	0.003	1.524	0.819	-	-
Plasma fatty acids (mmol/L)*	1.690	0.604	-	-	0.003	<0.001	0.006	<0.001	0.335	0.308	-	-
Plasma insulin (pmol/L)*	0.155	<0.001	-	-	1.774	0.003	-	-	0.238	0.155	-	-
HbA1c (%)	1.318	0.058	-	-	-0.489	0.004	-	-	-0.014	0.869	-	-
Malondialdehyde (μmol/L)	1.067	0.301	-	-	-0.182	0.012	-	-	0.001	0.997	-	-
Myocardial PCr/ATP	0.245	0.027	-	-	2.116	0.015	-	-	NA	NA	-	-
M/I value (10-mg·kg ⁻¹ ·min ⁻¹)/(pmol·L ⁻¹)*	0.136	<0.001	0.201	<0.001	1.164	<0.001	-	-	2.547	0.072	-	-
Liver triglyceride Content (%)*	NA	NA	-	-	0.074	0.001	-	-	0.479	0.027	-	-
MMRglu (10-nmol·mL ⁻¹ ·min ⁻¹)	0.706	0.001	-	-	NA	NA	-	-	0.109	0.015	0.109	0.015
MFAU (10-nmol·mL ⁻¹ ·min ⁻¹)	0.668	0.405	-	-	-1.073	0.072*	-	-	-0.351	0.312	-	-
MFAO (10-nmol·mL ⁻¹ ·min ⁻¹)	0.713	0.497	-	-	-1.051	0.088*	-	-	-0.390	0.264	-	-
Visceral fat volume (mL)*	15.668	<0.001	4.036	0.023	0.115	0.236	-	-	1.794	0.585	-	-
Subcutaneous fat volume (mL)	1.002	0.009	-	-	0.001	0.780	-	-	0.001	0.502	-	-
Plasma usCRP (mg/L)*	1.959	0.037	-	-	0.375	0.265	-	-	1.754	0.451	-	-
Body mass index (kg/m ²)	1.164	<0.001	-	-	-0.029	0.533	-	-	0.001	0.972	-	-

*For those independent variables, the regression coefficient is estimated for a difference of 1 U in the log of the independent variable. The transformed regression coefficient can be interpreted as follows: A difference of 1 unit log HbA1c is related to a 1.318 x higher liver triglyceride content. HbA1c, glycated haemoglobin; M/I value, whole body insulin sensitivity adjusted during the steady state. # MMRglu was significantly related with MFAU (P=0.010) and MFAO (P=0.009) in those patients in whom PCr/ATP was available. MMRglu, myocardial metabolic rate of glucose uptake; MFAU/O, myocardial fatty-acid uptake/oxidation; usCRP, ultrasensitive C-reactive protein

Hepatic triglyceride content and associations

According to the definitions, T2DM-high versus T2DM-low patients had higher median hepatic triglyceride content (14.4 (9.6-18.9) % versus 2.0 (1.2-3.8) %). Univariable and multivariable regression analyses of liver triglyceride content and myocardial metabolism are shown in Table 4, revealing M/I and visceral fat volume to be independently related to liver triglyceride content, plasma fatty-acid and lactate levels to be independently related to MMRglu and only MMRglu to be independently related to PCr/ATP. Amongst others, significant associations were seen between myocardial glucose and both fatty-acid metabolism and plasma levels of malondialdehyde. Correlations between liver triglyceride content, MMRglu and myocardial PCr/ATP ratio are shown in Figure 2.

Figure 2. Correlations between liver triglyceride content, myocardial metabolism and PCr/ATP ratio



Correlation between liver triglyceride content and myocardial metabolic rate of glucose (MMRglu) (A), between liver triglyceride content and myocardial phosphocreatine (PCr/ATP ratio) (B) and between MMRglu and myocardial PCr/ATP (C) in those T2DM patients in whom myocardial PCr/ATP was available

DISCUSSION

In the present study, reduced myocardial perfusion, glucose uptake and PCr/ATP ratio were found in T2DM patients with high liver triglyceride content with verified absence of inducible ischemia. LV function and dimensions, however, were similar as in T2DM patients with low liver triglyceride content. Moreover, liver triglyceride content was inversely associated with myocardial substrate and high-energy phosphate metabolism. Furthermore, to the best of

our knowledge, this study is the first to show a direct positive relationship between myocardial glucose metabolism and levels of myocardial PCr/ATP in human T2DM *in vivo*.

In the present study, T2DM patients with increased liver triglyceride content were characterized by lower HDL cholesterol, higher plasma triglyceride and CRP levels, and lower insulin sensitivity as compared with T2DM patient with low liver triglyceride content. None of the patients had CVD or diabetes-related complications, allowing the assessment of early myocardial abnormalities in the absence of potentially confounding effects of CAD and hypertension.

Myocardial glucose uptake was decreased in T2DM patients with high versus low liver triglyceride content, confirming previous data from Lautamaki et al. (12) who reported decreased PET-measured myocardial glucose consumption in non-stenotic myocardial segments of T2DM patients with CAD and high liver triglyceride content. In the present study, plasma fatty-acid levels were inversely related to myocardial glucose metabolism, therefore increased substrate levels may explain reduced myocardial glucose metabolism by reverse substrate competition. A more pronounced impairment of insulin signaling and reduced membrane bound GLUT-4 in T2DM patients with high liver triglyceride content, however, may also have contributed to decreased myocardial glucose metabolism (28,29).

Decreased resting myocardial perfusion and increased myocardial vascular resistance, observed in the present study, may indicate an early alteration in myocardial tissue and/or vascular properties in T2DM patients with high liver triglyceride content. Although age, gender and RPPP are known to influence resting myocardial perfusion (30), these were similar in the two groups, and therefore cannot explain the differences in myocardial perfusion between both groups. Induced hyperinsulinemia has been shown to significantly increase myocardial perfusion at rest in T2DM patients with CAD, also in non-affected cardiac regions, emphasizing the role of insulin action on myocardial perfusion (31). The observed difference in myocardial perfusion between both groups in the present study may be related to the same mechanism as T2DM patients with high liver triglyceride content were significantly more insulin resistant. Previously, resting myocardial perfusion under hyperinsulinemia in non-stenotic cardiac segments was not different in T2DM patients with high versus low liver triglyceride content and CAD, whereas myocardial glucose uptake was lower in patients with high liver triglyceride content (12). As myocardial perfusion and glucose metabolism were measured under fasting and hyperinsulinemic conditions, respectively, the present study design does not allow direct comparisons. Nevertheless, it is likely that the relatively small decrease in myocardial perfusion is responsible for only a minor fraction of the decrease in myocardial glucose metabolism in the T2DM patients with high liver triglyceride content. The major fraction of this decrease presumably is an intrinsic effect or related to substrate competition as mentioned above.

High liver triglyceride content in T2DM patients was also associated with a decreased myocardial PCr/ATP ratio. Myocardial PCr/ATP is known to be decreased in a variety of diseases,

among others obesity and T2DM (32-34). The present results in T2DM patients with verified absence of inducible ischemia are in line with a previous study by Perseghin et al.(11), who reported a reduced myocardial PCr/ATP ratio in young non-diabetic men with high versus low liver triglyceride content. Fatty-acids, glucose and lactate are the primary energy substrates of the heart, with a substrate preference depending on myocardial workload and substrate supply in conjunction with feeding status (35). Glucose, however, is the most energy efficient substrate (35). Experimental data suggest that increased fatty-acid relative to glucose metabolism leads to the formation of toxic lipid signaling molecules and oxidative stress, ultimately resulting in mitochondrial dysfunction and reduced ATP synthesis (4,36). In the present study inverse relationships between myocardial glucose and fatty-acid metabolism and plasma levels of malondialdehyde were found. Moreover, a positive relationship between myocardial glucose metabolism and myocardial PCr/ATP ratio was found in T2DM patients. The present study design precludes an assessment as to whether those associations describe a cause and effect relationship. It may, however, be speculated that when glucose relative to fatty-acid metabolism contributes more to total ATP synthesis, this may result in a more favorable myocardial energy level.

Although myocardial metabolism differed between T2DM patients with high and low liver triglyceride content, no such differences were seen in myocardial systolic and diastolic function or dimensions. This finding corresponds with the earlier work of Perseghin et al. (11), who found differences in PCr/ATP ratios, but not in myocardial function and dimensions between non-diabetic men with high versus low liver triglyceride content.

The following limitations need to be considered in the present study. Firstly, PET measurements were performed under different conditions, i.e. fasting perfusion and fatty-acid measurements, but euglycemic hyperinsulinemic clamp conditions for measurement of glucose metabolism. A euglycemic hyperinsulinemic clamp is mandatory, as under fasting conditions virtually no glucose metabolism will be present in insulin resistant myocardium. Differences in myocardial perfusion and glucose metabolism in the two groups are therefore not readily interpretable. Secondly, there was substantially less myocardial PCr/ATP data available than for the other measurements. Thirdly, only men were included, which limits generalizability of the results to both genders.

In conclusion, in the absence of diabetes related complications and inducible ischemia, T2DM patients with high liver triglyceride content showed decreased myocardial perfusion, glucose uptake, high-energy phosphate metabolism and whole-body insulin resistance compared with similar patients with low liver triglyceride content. The long-term clinical implications of this association between liver steatosis and altered cardiac metabolism require further study in T2DM.

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