Liver fat content in type 2 diabetes: relationship with hepatic perfusion and substrate metabolism

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Short title: Liver physiology and triglyceride content in diabetes

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Objective. Hepatic steatosis is common in type 2 diabetes mellitus (T2DM). It is causally linked to the features of the metabolic syndrome, liver cirrhosis and cardiovascular disease. Experimental data have indicated that increased liver fat may impair hepatic perfusion and metabolism. The aim of the present study was to assess hepatic parenchymal perfusion, together with glucose and fatty-acid metabolism in relation to hepatic triglyceride content.

Research Design and Methods. Fifty-nine men with well controlled T2DM and 18 age matched healthy normoglycemic men were studied using positron emission tomography (PET) to assess hepatic tissue perfusion, insulin stimulated glucose and fasting fatty-acid metabolism respectively in relation to hepatic triglyceride content, quantified by proton magnetic resonance (MR) spectroscopy. Patients were divided into two groups with hepatic triglyceride content below (T2DM-low) or above (T2DM-high) the median of 8.6%.

Results. T2DM-high patients had the highest BMI, HbA1c and lowest whole-body insulin sensitivity (ANOVA, all *P*<0.001). Compared to controls and T2DM-low patients, T2DM-high patients had the lowest hepatic parenchymal perfusion (*P*=0.004) and insulin-stimulated hepatic glucose uptake (*P*=0.013). The observed decrease in hepatic fatty acid influx rate constant, however, only reached borderline significance (*P*=0.088). In T2DM patients, hepatic parenchymal perfusion (r= -0.360, *P*=0.007) and hepatic fatty acid influx rate constant (r= -0.407, *P*=0.007) correlated inversely with hepatic triglyceride content. In a pooled analysis, hepatic fat correlated with hepatic glucose uptake (r= -0.329, *P*=0.004).

Conclusions. In conclusion, T2DM patients with increased hepatic triglyceride content showed decreased hepatic parenchymal perfusion and hepatic insulin mediated glucose uptake, suggesting a potential modulating effect of hepatic fat on hepatic physiology.

Obesity and type 2 diabetes (T2DM) have grown to epidemic proportions in virtually all parts of the world due to a sedentary lifestyle and positive energy balance.(1) Hepatic steatosis is a common finding in T2DM, which is causally linked to features of the metabolic syndrome, liver cirrhosis and cardiovascular disease.(2;3) The pro-atherogenic serum lipid profile associated with hepatic steatosis is a consequence of an increased synthesis of very low density lipoproteins (VLDL).(4) Moreover, hepatic steatosis is associated with impaired insulin signaling in insulin responsive tissues by promoting the formation of humoral factors,(5) and it plays a role in atherogenesis via induction of systemic inflammation.(6) The liver is the central organ for lipid and glucose metabolism, both of which are additionally regulated by insulin.(7-9) Liver steatosis is associated with impaired inhibition of hepatic glucose output, but also with impaired insulin clearance.(10;11) Using splanchnic catheterization in patients with T2DM and healthy controls, glucose and fatty-acid fluxes into the liver have been characterized.(12-15) However, those techniques can not discriminate between the effects of the liver versus those of the other splanchnic tissues. More recently, positron emission tomography (PET) was introduced to non-invasively assess hepatic substrate fluxes.(16-18) To date, however, only a few studies have addressed effects of gluco-metabolic disorders on hepatic disposal of
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Hepatic steatosis has also been associated with alterations of hepatic hemodynamics. Using non-invasive Doppler sonography, decreased portal vein hemodynamics were demonstrated in patients with fatty liver disease.(22;23) Human donor livers, studied during organ retrieval using laser Doppler flowmetry, showed diminished microcirculation compared with control livers.(24) Moreover, animal data revealed that graded steatosis decreased parenchymal microcirculation.(25) In addition to these highly invasive methods, non-invasive in vivo studies of hepatic perfusion have also been performed using PET.(26-28) However, little is known about the relationship between liver triglyceride content with hepatic perfusion or substrate metabolism in human T2DM.

The purpose of the present study was to measure hepatic perfusion and metabolism and to investigate the relationship with hepatic fat content in T2DM patients without diabetes related complication and age matched healthy male subjects.

METHODS

Participants. Fifty-nine T2DM patients and 18 healthy controls participated in this two-center study, which was approved by the Medical Ethics Review Committees of both centers and performed in compliance with the Declaration of Helsinki. All subjects signed informed consent prior to inclusion. Patients and controls were recruited by advertisements in local papers. Male T2DM patients, aged 45-65 years, without diabetes related complications were eligible. Inclusion criteria were glycated hemoglobin A1c (HbA1c) 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). In addition, only moderate alcohol intake was allowed. Patients were excluded if they had a history of or current hepatic or cardiovascular disease. Other exclusion criteria were the use of insulin, fibrates, thiazolidinediones or other hormonal replacement therapy. Healthy males, aged 45-65 years, with normal glucose metabolism, as assessed by a 75 g oral glucose tolerance test, were eligible as controls. Inclusion criteria were BMI of 25-32 kg/m² and blood pressure below 150/85 mm Hg. Patients and healthy controls underwent a screening consisting of medical history, physical examination, electrocardiogram and fasting blood- and urine analyses. In addition, patients underwent dobutamine-stress echocardiography to confirm absence of inducible ischemia. All eligible patients entered a 10 week run-in period in which their blood glucose lowering agents were stopped. Subsequently all patients were transferred to a comparable dosing of glimepiride monotherapy. Data on myocardial perfusion and substrate uptake have previously been published elsewhere.(29)

Study design. The study protocol was performed during two visits, within the same week. At one of the visits, hepatic triglyceride content was measured using 1H-MRS. In addition, subcutaneous and visceral fat volumes were measured using MRI. At the other visit, hepatic perfusion and metabolism were measured using PET. At both occasions, patients visited the clinical research unit in the morning at 08:00 following an overnight fast of approximately 12-15 hours and no glucose-lowering agents were taken on the day of the assessments.

Magnetic resonance imaging and spectroscopy. All MR studies were performed at a single center (Leiden) on the same 1.5 Tesla whole body MR scanner (Gyrosan ACS/NT15, Philips, Best, the Netherlands) with subjects at rest and in supine position. Hepatic 1H-MR spectra were obtained as described previously.(30) In short, 1H-MRS of the liver was performed with an 8 ml voxel positioned in the right lobe of the liver.
avoiding gross vascular structures and adipose tissue depots. Sixty-four averages were collected with water suppression. Spectra were obtained with an echo time (TE) of 26 ms and a repetition time (TR) of 3000 ms. 1024 data points were collected using a 1000 Hz spectral. Without changing any parameter, spectra without water suppression, with a TR of 10 s and with 4 averages were obtained as an internal reference. 1H-MRS data were fitted using Java-based MR user interface software (jMRUI version 2.2, Leuven, Belgium), as described previously.(31) Hepatic triglyceride content relative to water was calculated as 100 · (signal amplitude of triglyceride)/(signal amplitude of water). T2DM patients were divided according to the median liver fat content in a low (≤ 8.6 %; T2DM-low) and high (> 8.6 %; T2DM-high) liver triglyceride group. Abdominal visceral and subcutaneous fat depots were quantified using MRI.(32) A turbo spin echo imaging protocol was used and imaging parameters included the following: TE = 11 ms, TR = 168 ms, flip angle = 90º, slice thickness 10 mm. Three consecutive transverse images were obtained during 1 breath hold, with the middle image at a level just above the fifth lumbar vertebra. The volumes of the visceral and subcutaneous fat depots of all slices were calculated by converting the number of pixels to square centimeters multiplied by the slice thickness. The total volume of the fat depots was calculated by summing fat volumes of all three slices.

**Positron emission tomography.** All PET studies were performed at a single center (Amsterdam) using an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA). Patients received three venous catheters; one in both antecubital veins and one in a hand vein being wrapped into a heated blanket to obtain arterialized blood during the [18F]FDG scan. Hepatic tissue perfusion was performed in 2D mode and quantified using [15O]H2O (1100 MBq). Hepatic glucose and fatty-acid uptake were performed in 3D mode and quantified using [18F]FDG (170 MBq) and [11C]palmitate (185 MBq), respectively. Perfusion and fatty-acid uptake were assessed in the postabsorptive state, whereas glucose uptake was performed under hyperinsulinemic euglycemic conditions. The following scan protocol was used for all studies. Following a 10 minutes transmission scan for attenuation correction, [15O]H2O was injected and a 10 minutes dynamic emission scan, consisting of 40 frames with progressively increasing frame length, was acquired. Subsequently, a 30 minutes dynamic emission scan, consisting of 34 frames with progressively increasing frame length, was performed following [11C]palmitate injection. Next, a euglycemic hyperinsulinemic clamp procedure was started using an insulin infusion rate of 40 mU·m⁻²·min⁻¹ as previously described,(33) Euglycemia was maintained by adapting the glucose infusion rate to maintain a plasma glucose level of 5 mmol/l. Whole body insulin sensitivity (M/I value) was calculated as the mean plasma glucose level between 90-120 minutes from start of the clamp procedure and then divided by the mean plasma insulin levels in the same time interval. The insulin clearance rate was estimated by dividing the exogenous insulin infusion rate by the steady-state plasma insulin concentrations during the clamp. Under these conditions, the described ratio corresponds to the metabolic clearance rate of systemically administered insulin, minus a small (though variable) part represented by residual insulin secretion. The posthepatic insulin delivery rate of insulin is then calculated as the product of the insulin clearance rate and fasting plasma insulin levels. At steady state (approximately 90 minutes after start of clamp) and following a second transmission scan, [18F]FDG was injected and a 60 minutes dynamic emission of 40 frames with progressively increasing frame length 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, \(^{11}\)CO\(_2\) was measured during the \[^{11}\text{C}\]palmitate scan.(29;34)

**PET data analysis.** Emission data were corrected for physical decay of the respective tracers and for dead time, scatter, randoms and photon attenuation. In order to generate myocardial time-activity curves, large regions (2 by 5 cm) of interest (ROIs) were defined in the right lobe of the liver on 4-5 consecutive planes of OSEM reconstructed (summed) images and then copied to the three dynamic images to obtain one tissue time-activity curve per tracer for each subject. Additionally, circular ROIs (15 mm \(\varnothing\)) were drawn on 10 consecutive planes on the respective dynamic images in the aorta ascendens and grouped to obtain one input function (IDIF) for each tracer.

Finally, delay \(\Delta t\), dispersion constant \(k_g\), \(V_T\), \(F_A\), \(F_P\), and fractional hepatic blood volume \(V_B\), were determined by non-linear regression using the following operational equation in which the right hand side of eq (2) was substituted for \(C_P(t)\):

\[
C_P(t) = C_A(t) - \frac{k_g e^{-k_g t}}{V_T} \otimes e^{-k_g t}
\]  

(2)

The \(K_i\) of \[^{11}\text{C}\]palmitate was not multiplied by fasting fatty-acid levels, as these may not accurately reflect portal vein concentrations, hence only \(K_i\) is provided. Patlak analysis of \[^{11}\text{C}\]palmitate was confined to the interval from 3 to 10 minutes after tracer injection, as a previous study in the liver has shown that labeled triglyceride metabolites of \[^{11}\text{C}\]palmitate become detectable after 10 minutes.(35) Although for this time interval no correction for labeled triglycerides was necessary, a correction of \[^{11}\text{C}\]palmitate IDIFs for \[^{11}\text{C}\]CO\(_2\) was still performed, as described elsewhere.(29;34) In addition, the validity of using the Patlak method for analyzing \[^{11}\text{C}\]palmitate data was assessed using spectral analysis.(36) Spectral analysis allows for 1) assessment of the number of tissue compartments identifiable in the data,
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and 2) whether these compartments represent irreversible or reversible tracer kinetics, without prior assumptions about the underlying tracer kinetics. Use of this approach showed the validity of the Patlak method, as in all scans only one irreversible compartment was detected for the time interval selected (data not shown).

**Biochemical analyses.** Samples were analyzed at one certified central laboratory (Amsterdam). Plasma glucose was quantified using a hexokinase based technique (Roche Diagnostics, Mannheim, Germany). HbA1c was determined by high-performance liquid chromatography (HPLC; Menarini Diagnostics, Florence, Italy; reference values: 4.3-6.1%). 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 free-fatty acids were measured by an enzyme-linked immunosorbent assay (Wako Chemicals, Neuss, Germany). Plasma insulin levels were quantified by an immunoradiometric assay (Bayer Diagnostics, Mijdrecht, The Netherlands). 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 coefficient of variation (CV) 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.(37) The intra-assay CV was 5.7%.

**Statistical analyses** Values are expressed as mean±standard error or as median (interquartile range) in case of skewed distribution. Non-normally distributed data were log-transformed. Comparisons between controls, T2DM-low and T2DM-high patients were performed using Analysis of Variance (ANOVA), including the Bonferroni posthoc multiple comparisons test. Pearson’s and Spearman’s (where appropriate) univariate correlation coefficients were calculated and linear regression was used to control for covariates. Statistical analysis was performed using SPPS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). A two-tailed probability value <0.05 was considered significant.

**RESULTS**


**Subject characteristics.** Baseline characteristics of patients, categorized according to liver fat content, and controls are listed in Table 1. All groups were similar with respect to age, and both T2DM groups had comparable disease duration and medication use. As expected, anthropometric and hemodynamic parameters (which were all in the normal range) differed significantly among groups. Plasma lipid profiles and liver enzymes were different between groups (Table 1). Metabolic characteristics under postabsorptive and hyperinsulinemic conditions are displayed in Table 2 and showed differences between groups. Plasma fatty-acids (postabsorptive state) and plasma lactate (hyperinsulinemia), however, were similar between groups.

**Hepatic and abdominal fat.** T2DM-high patients had, compared with T2DM-low patients and controls, the highest hepatic triglyceride content: 21.6 (12.9-29.4) versus 2.6 (1.5-5.2) and 2.5 (1.0-4.2)% respectively, ANOVA P<0.001. Figure 1 shows a representative MRI image and spectrogram. Subcutaneous and visceral fat volumes were statistically different between groups (736±47 versus 572±39 and 598±52.
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ml, \(P=0.020\) and (440 (333-578) versus 318 (248-404) and 264 (203-340) ml, \(P<0.001\), respectively.

**Hepatic parenchymal perfusion and substrate uptake.** Figure 2 shows representative OSEM (summed) reconstructed PET images of the liver for \([^{11}\text{C}]\)palmitate, \([^{18}\text{F}]\)FDG and fits on the data for the respective tracers. T2DM-high patients had, compared with T2DM-low patients and controls, the lowest hepatic perfusion (0.647±0.038 versus 0.795±0.042 and 0.850±0.047 ml \(\cdot\) ml\(^{-1}\) \(\cdot\) min\(^{-1}\), ANOVA \(P=0.004\), Figure 3A). T2DM-high patients had, compared with T2DM-low patients and controls, the lowest insulin mediated HGU (20.4±1.9 versus 24.1±2.1 and 30.7±3.0 µmol \(\cdot\) ml\(^{-1}\) \(\cdot\) min\(^{-1}\), respectively, \(P=0.013\), Figure 3B). No tracer loss from the liver could be detected during scan time. The mean hepatic fatty-acid influx rate constant (Figure 3C) was lower in T2DM-high patients compared with T2DM-low patients and controls, but only reached borderline significance (\(P=0.088\)).

**Correlations between hepatic fat content, parenchymal perfusion and substrate uptake.** In a pooled analysis, hepatic triglyceride content correlated inversely with hepatic perfusion (\(r=-0.402, P=0.001\); Figure 3A) and hepatic fatty-acid influx rate constant (\(r=-0.335, P=0.004\); Figure 3B), which both remained significant after correction for diabetic status, HbA1c, BMI, visceral fat content, plasma fatty-acid and lactate levels. Hepatic triglyceride content also correlated inversely with HGU (\(r=-0.329, P=0.004\); Figure 3C), which remained significant after correction for diabetic status, HbA1c, BMI and visceral fat content, but not when additionally correcting for plasma fatty-acid or lactate. Hepatic triglyceride content, but not hepatic perfusion or hepatic fatty-acid influx rate constant, were correlated with M/I value (\(r=-0.684, P<0.001\)), malondialdehyde (\(r=0.427, P<0.001\)), usCRP (\(r=0.326, P=0.005\)), insulin clearance rate (\(r=-0.459, P<0.001\)), visceral (\(r=0.612, P<0.001\)) and subcutaneous fat volume (\(r=0.392, P<0.001\)). The hepatic glucose influx rate constant correlated inversely with plasma fatty-acid levels (\(r=-0.246, P=0.036\), HbA1c (\(r=-0.310, P<0.007\)) and malondialdehyde (\(r=-0.434, P<0.001\)).

In T2DM patients alone, hepatic fat content correlated inversely with hepatic perfusion (\(r=-0.360, P=0.007\)) and hepatic fatty-acid influx rate constant (\(r=-0.407, P=0.007\)), whereas borderline significant associations were found with HGU (\(r=-0.245, P=0.057\)). Hepatic fat content, but not hepatic perfusion or hepatic fatty-acid influx rate constant, correlated with M/I value (\(r=-0.657, P<0.001\)) and usCRP (0.375, \(P=0.005\)), insulin clearance rate (\(r=-0.436, P=0.001\)), visceral (\(r=0.540, P<0.001\)) and subcutaneous fat (\(r=0.375, P=0.003\)) volumes. The hepatic glucose influx rate constant rate, inversely correlated with malondialdehyde (\(r=-0.380, P=0.004\)) and borderline with plasma fatty-acids (\(r=-0.251, P=0.059\)). None of these correlations were observed in controls alone.

**DISCUSSION**

Using MRS and PET in the same patients, the present study provides evidence for a potential modulating effect of hepatic fat content on hepatic physiology in T2DM patients. Reduced hepatic parenchymal perfusion, insulin-mediated HGU and a borderline decrease in hepatic fatty-acid influx rate constant were observed in T2DM patients with increased hepatic triglyceride content. Moreover, hepatic triglyceride content was directly and inversely related to hepatic perfusion, hepatic glucose and fatty-acid metabolism.

**Hepatic fat content and relationship with hepatic parenchymal perfusion.** Although flow through portal vein and hepatic artery is readily accessible using Doppler sonography,(22;23) in vivo studies on human
hepatic (parenchymal) perfusion are limited due to the often (highly) invasive methodology required. Indirect methods for measuring hepatic blood flow have been used and include the assessment of clearance or dilution of a dye or marker (gas or microspheres), which have a wider range of clinical applicability than the direct methods.(38) Moreover, non-invasive measurements of hepatic perfusion using PET with the freely diffusible flow tracer $[^{15}O]H_2O$ have been shown to provide reliable estimates of hepatic blood flow, when taking into account the dual input from hepatic artery and vena porta.(27;28) In the present study decreased hepatic parenchymal perfusion was observed in T2DM patients with increased liver triglyceride content, but not in those T2DM patients with low liver triglyceride content, as compared with controls, implying a potential modulating effect of liver fat per se.

These results extend data from previous studies suggesting a modulating effect of increased hepatic fat content on hepatic blood flow velocity and perfusion. It has been shown that the level of fatty infiltration in humans alters portal vein hemodynamics in a graded way.(22;23) Especially under stress conditions, such as during ischemia-reperfusion or transplantation, the fatty liver has shown decreased adaptability and hence increased risk of failure.(39) In addition to changes in hepatic macrocirculation, alterations in the hepatic microvasculature have been implicated. In steatotic livers of human donors, laser Doppler flowmetry revealed a significant decrease in hepatic parenchymal perfusion.(24) In New Zealand white rabbits with diet-induced hepatic steatosis, Seifalian et al(25) found that graded steatosis progressively reduced hepatic blood flow velocity and hepatic parenchymal perfusion. Moreover, they observed an inverse correlation between degree of fat infiltration and both total hepatic blood flow and the hepatic parenchymal perfusion was found, with the biggest effect on the latter.

The mechanisms by which increased liver fat affect hepatic perfusion include factors like structural changes in the liver, a microvascular inflammatory response and possibly vascular insulin resistance. Experimental studies in several animal models of diet and genetically induced hepatic steatosis have shown that reductions in sinusoidal perfusion are initially due to enlarged hepatic parenchymal cells overloaded with lipids.(40-43) Consequently, parenchymal cell plates become wider, which results in narrowing and deformation of the lumen of sinusoids, reducing their volume. This eventually leads to sinusoidal dysfunction and impaired hepatic perfusion.(42) Increased leukocyte adherence to endothelial cells, expression of adhesion molecules and upregulation of NF-kappaB have been shown to promote reactive oxygen species (ROS) generation, with subsequent inflammation and formation of vasoactive metabolites, all of which may be implicated in decreased hepatic parenchymal perfusion.(44) Moreover, insulin resistance, one of the hallmarks of T2DM pathology and strongly associated with hepatic steatosis, may additionally decrease hepatic microcirculatory flow by impaired insulin receptor signaling via the PI3-kinase/Akt/eNOS cascade, which in turn may result in decreased nitric oxygen (NO) synthesis by endothelial cells and hence decreased NO mediated vasodilation.(44;45) In addition, stimulated signaling through the insulin-receptor mediated MAPK/ERK pathway may additionally favor vasoconstriction and abnormal angiogenesis, contributing to impaired microvascular hepatic perfusion.(46) Although in the present study no direct relationships were found between hepatic parenchymal perfusion and whole-body insulin sensitivity, oxidative stress or usCRP, the hepatic parenchymal
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perfusion was inversely correlated with hepatic fat content. Therefore, more studies are warranted to further explore these interrelations.

**Hepatic triglyceride content and relationship with substrate metabolism.** Interestingly, only a borderline significant difference was found in the fasting hepatic fatty-acid influx rate constant across groups, caused by the lower uptake rate in T2DM-high, but not T2DM-low patients. Depending on the condition, fatty-acid extraction or uptake has been reported to be unaltered (13;14), decreased (15;19) or increased.(14;47) Using PET with the fatty-acid analogue tracer 14(R,S)-¹⁸F-fluoro-6-thia-heptadecanoic acid, Iozzo et al found decreased fatty-acid extraction in 10 fasting patients with impaired glucose tolerance (IGT) compared with 8 healthy controls.(19) These findings were primarily explained by reverse substrate competition, as plasma glucose sampled from arterialized blood correlated inversely with fatty-acid uptake. In the present study, during the [¹¹C]palmitate PET scan, only venous sampling was performed and hence this relation could not be tested reliably.

In the present study previous findings were confirmed, indicating that both T2DM and liver fat content are inversely related to insulin-stimulated hepatic glucose-uptake.(20;48) Hepatic glucose influx and output are directly regulated by insulin through several enzymes. Insulin initializes the up-regulation of glucokinase and glycogen synthase and conversely inhibits glucose-6-phosphatase and glycogen phosphorylase in hepatocytes.(8) In hepatic insulin resistance, impaired activity of these key enzymes may therefore lead to decreased insulin-stimulated HGU.(49)

An indirect mechanism underlying the negative relationship between liver fat and HGU may be increased fatty-acid fluxes related to increased lipolysis from insulin-resistant adipose tissue. The inverse association between plasma fatty-acids and HGU rate is in line with this assumption. Furthermore, other studies have shown that a combined intralipid/heparin infusion increased plasma fatty-acids and reduced splanchnic and peripheral glucose uptake in T2DM patients.(50) Moreover, although the present study is aimed at HGU, it should be mentioned that hepatic glucose uptake only constitutes a small percentage of net change in glucose metabolism during the clamp.

Finally, the liver is the main site involved in insulin clearance and degradation.(51) Recently, Kotronen et al.(11) found that increased hepatic fat was associated with impaired insulin clearance in 80 nondiabetic subjects. The present inverse relationship between liver fat content and insulin clearance is in line with those results.

**Limitations.** In the present study, we used ¹H-MRS to measure hepatic triglyceride content. To that purpose, only three MR slides of the liver were made for voxel localization and hence total liver volume could not be calculated. Thus the study's conclusions are limited to liver tissue studied within the volume of the voxel. Although liver volume was probably increased in the T2DM-high group, the effect of an increased liver volume on our findings cannot be established. From animal studies, however, it seems less likely that an increase in liver volume substantially influenced our findings (25;52). In addition to a decrease in the total number of hepatocytes, many structural changes in the fatty liver may negatively influence hepatic metabolism and parenchymal perfusion.

**CONCLUSION**

T2DM patients with high liver triglyceride content have a poorer metabolic profile than age-matched controls and T2DM patients with a liver triglyceride content in the normal range. In addition, T2DM patients with high liver triglyceride content show decreased hepatic parenchymal perfusion and insulin-
mediated glucose uptake. Finally, hepatic triglyceride content is inversely related to hepatic parenchymal perfusion, HGU and hepatic fatty-acid influx rate constant suggesting a potential modulating effect of hepatic fat on hepatic physiology.

Author contributions. LJR: Conception and design of the study, analysis and interpretation of data. Drafting and revising the manuscript. RWM: Conception and design of the study, analysis and interpretation of data. Revising the manuscript. ML: Analysis and interpretation of data. Modeling of PET data and technical assistance. Drafting and revising the manuscript.

HJL: Conception and design of the study. Revising the manuscript. JAR: Conception and design of the study. Revising the manuscript. AR: Conception and design of the study. Revising the manuscript. JWT: Analysis and interpretation of the study. Revising the manuscript. RJH: Conception and design of the study. Revising the manuscript. AAL: Conception and design of the study. Revising the manuscript. JWAS: Conception and design of the study. Revising the manuscript. MD: Conception and design of the study, analysis and interpretation of data. Drafting, co-writing and revising the manuscript.

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Conflict of interest disclosures. Dr. Diamant reports receiving consulting and lecture fees from Eli Lilly, Merck, Novartis, Pfizer and Sanofi and research grants from Eli Lilly, Merck, Novartis, Novo Nordisk and Glaxo Smith Kline. Dr. Heine is employed by Eli Lilly & company as of January 2008.

Figure Legends:

Figure 1.
Representative images of OSEM (summed) reconstructed PET images of the liver with $[^{11}C]$palmitate (A) and $[^{18}F]$FDG (B) with ROIs used for analysis indicated. Images show uptake in the liver on the left and uptake in the heart on the upper-right. Time course of $[^{15}O]$H$_2$O concentration (C) in the liver (circles), with hepatic perfusion model fit (straight line). Patlak plots of $[^{11}C]$palmitate (D) and $[^{18}F]$FDG (E) data, respectively. The blue dots in Figure 2 D&E were excluded from the analysis. The slope of the linear fits equals the net rate of influx $K_i$. Note the correspondence between the respective $K_i$ values and the uptake seen in figures A and B, which is much higher for $^{11}$C-palmitate (fasting state) than for $^{18}$F-FDG (hyperinsulinemic state).

Figure 2.
Hepatic perfusion (A), hepatic glucose uptake (HGU) (B), hepatic fatty-acid influx rate constant (C), in controls (□).T2DM patients with low liver triglyceride content (■) and T2DM patients with high liver triglyceride content (■). $K_i$ = hepatic influx rate constant. $P$ values are from Bonferroni post-hoc analysis. For $P$ ANOVA see text.

Figure 3.
Correlations between hepatic triglyceride content % and hepatic perfusion (A), hepatic fatty-acid influx rate constant (B), hepatic glucose uptake (HGU) (C), in pooled analysis of T2DM patients and controls.

REFERENCES
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30. van der Meer,RW, Hammer,S, Lamb,HJ, Frolich,M, Diamant,M, Rijzewijk,LJ, de,RA, Romijn,JA, Smit,JW: Effects of short-term high-fat, high-energy diet on hepatic and


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Figure 1.

A

B

C

D

E

$K_1 = 0.184$

$K_1 = 0.00565$
Figure 2.

A

Hepatic perfusion (ml·m⁻²·min⁻¹)

\[ P = 0.007 \]

\[ P = 0.031 \]

Controls  T2DM low  T2DM high

B

HGU (µmol·gr⁻¹·min⁻¹)

\[ P = 0.010 \]

\[ P = 0.161 \]

Controls  T2DM low  T2DM high

C

HK₁¹⁵C-palmitate (min⁻¹)

Controls  T2DM low  T2DM high
Figure 3.

![Graph 1: Hepatic perfusion vs. LOG hepatic triglyceride %](image1)

- Hepatic perfusion (ml. ml⁻¹ min⁻¹)
- LOG hepatic triglyceride %
- r = -0.402
- P = 0.001

![Graph 2: HKᵢ "C-palmitate" vs. LOG hepatic triglyceride %](image2)

- HKᵢ "C-palmitate" (min⁻¹)
- LOG hepatic triglyceride %
- r = -0.335
- P = 0.004

![Graph 3: HGU vs. LOG hepatic triglyceride %](image3)

- HGU (µmol gr⁻¹ min⁻¹)
- LOG hepatic triglyceride %
- r = -0.329
- P = 0.004
Table 1. Subject characteristics

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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.3 ± 0.6</td>
<td>26.7 ± 0.5</td>
<td>30.0 ± 0.5    #&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>2.1 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>0.085</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>102 ± 2</td>
<td>99 ± 2</td>
<td>107 ± 2</td>
<td>0.005</td>
</tr>
<tr>
<td>Systolic bloodpressure, mm Hg</td>
<td>118 ± 3</td>
<td>124 ± 2</td>
<td>130 ± 2</td>
<td>0.002</td>
</tr>
<tr>
<td>Diastolic bloodpressure, mm Hg</td>
<td>72 ± 2</td>
<td>73 ± 1</td>
<td>78 ± 1       #&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>56 ± 2</td>
<td>64 ± 2 *</td>
<td>66 ± 1       #&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Metabolic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA₁c, %</td>
<td>5.4 ± 0.1</td>
<td>7.0 ± 0.2 *</td>
<td>7.3 ± 0.2 *   #&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>5.0 ± 0.2</td>
<td>4.3 ± 0.1 *</td>
<td>4.5 ± 0.2</td>
<td>0.006</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>3.2 ± 0.1</td>
<td>2.6 ± 0.1 *</td>
<td>2.7 ± 0.7 #&lt; 0.007</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.24 (1.10-1.63)</td>
<td>1.05 (0.85-1.29) *</td>
<td>0.96 (0.82-1.09) #&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>0.8 (0.6-1.2)</td>
<td>1.1 (0.8-1.6) *</td>
<td>1.8 (1.2-2.3) #&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>25 (18-33)</td>
<td>26 (21-33)</td>
<td>37 (30-51)    #&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>AST, U/l</td>
<td>24 (20-30)</td>
<td>28 (21-36)</td>
<td>28 (24-38)    0.139</td>
<td></td>
</tr>
<tr>
<td>γ-GT, U/l</td>
<td>23 (17-29)</td>
<td>23 (18-37)</td>
<td>42 (35-48)    #&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>usCRP, mg/l</td>
<td>3.0 (1.7-6.3)</td>
<td>2.9 (1.6-4.5)</td>
<td>4.7 (3.5-6.8) #&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Malondialdehyde, µmol/l</td>
<td>6.0 ± 0.1</td>
<td>9.7 ± 0.5 *</td>
<td>10.0 ± 0.4 *  #&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Medications – % (n/N)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>NA</td>
<td>35 (8/23)</td>
<td>47 (17/36)</td>
<td>0.423</td>
</tr>
<tr>
<td>Any antihypertensive medication</td>
<td>NA</td>
<td>35 (8/23)</td>
<td>47 (17/36)</td>
<td>0.423</td>
</tr>
</tbody>
</table>

NOTE: Data are mean ± SE, median (IQR). N.A., not applicable. T2DM-low = T2DM patients with liver triglyceride content ≤ 8.6 %. T2DM-high = T2DM patients with liver triglyceride content > 8.6 %. # = significant difference between controls and T2DM-high. * = significant difference between controls and T2DM-low. $ significant difference between T2DM groups. HbA₁c = glycated hemoglobin. LDL = low-density lipoprotein. HDL = high-density lipoprotein. ALT = alanine aminotransferase. AST = aspartate aminotransferase. γ-GT = γ-glutamyl transferase. usCRP = ultra sensitive C-reactive protein.
Table 2. Metabolic characteristics in controls and type 2 diabetic patients with low and high hepatic triglyceride content

<table>
<thead>
<tr>
<th>Metabolic characteristic (fasting state)</th>
<th>Controls</th>
<th>T2DM-low</th>
<th>T2DM-high</th>
<th>P value ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>5.2 (4.9-5.4)</td>
<td>8.3 (6.7-10.1) *</td>
<td>8.0 (7.1-8.7) #</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Plasma non-esterified fatty acids, umol/l</td>
<td>470 (360-540)</td>
<td>450 (410-570)</td>
<td>500 (370-590)</td>
<td>0.624</td>
</tr>
<tr>
<td>Plasma lactate, mmol/l</td>
<td>0.8 (0.7-0.9)</td>
<td>1.1 (0.9-1.3) *</td>
<td>1.2 (1.0-1.5) #/$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>28 (19-33)</td>
<td>39 (28-62) *</td>
<td>78 (62-99) #/$</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metabolic characteristics (hyperinsulinemic state)</th>
<th>Controls</th>
<th>T2DM-low</th>
<th>T2DM-high</th>
<th>P value ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma non-esterified fatty acids, umol/l</td>
<td>40 (20-48)</td>
<td>50 (30-85) *</td>
<td>115 (70-173) #/$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Plasma lactate, mmol/l</td>
<td>1.1 (0.9-1.3)</td>
<td>1.0 (0.9-1.2)</td>
<td>1.1 (1.0-1.4)</td>
<td>0.560</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>511 ± 67</td>
<td>513 ± 23</td>
<td>643 ± 26 #/$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>M/I value, mg/(kg·min)/(pmol/l)</td>
<td>1.13 (0.73-1.66)</td>
<td>0.68 (0.46-1.0) *</td>
<td>0.37 (0.17-0.45) #/$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Insulin clearance rate, ml/min</td>
<td>1101 (1017-1270)</td>
<td>1029 (951-1262)</td>
<td>945 (816-1053) #/$</td>
<td>0.003</td>
</tr>
<tr>
<td>Post-hepatic insulin delivery rate, pmol/min</td>
<td>29 (21-40)</td>
<td>48 (30-77) *</td>
<td>78 (56-97) #/$</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

NOTE: Data are mean ± SE, median (IQR). T2DM-low = T2DM patients with liver triglyceride content ≤ 8.6 %. T2DM-high = T2DM patients with liver triglyceride content > 8.6 %. # = significant difference between controls and T2DM-high. * = significant difference between controls and T2DM-low. $ significant difference between T2DM groups. M value = whole body insulin sensitivity. M/I value = M value adjusted for insulin during the steady state.