Liver Fat Content in Type 2 Diabetes: Relationship With Hepatic Perfusion and Substrate Metabolism

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OBJECTIVE—Hepatic steatosis is common in type 2 diabetes. 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 current 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 type 2 diabetes and 18 age-matched healthy normoglycemic men were studied using positron emission tomography 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 spectroscopy. Patients were divided into two groups with hepatic triglyceride content below (type 2 diabetes-low) or above (type 2 diabetes-high) the median of 8.6%.

RESULTS—Type 2 diabetes-high patients had the highest BMI and A1C and lowest whole-body insulin sensitivity (ANOVA, all P < 0.001). Compared with control subjects and type 2 diabetes-low patients, type 2 diabetes-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 type 2 diabetic 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, type 2 diabetic 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. Diabetes 59:2747–2754, 2010

Obesity and type 2 diabetes have grown to epidemic proportions in virtually all parts of the world because of a sedentary lifestyle and positive energy balance (1). Hepatic steatosis is a common finding in type 2 diabetes, which is causally linked to features of the metabolic syndrome, liver cirrhosis, and cardiovascular disease (2,3). The proatherogenic serum lipid profile associated with hepatic steatosis is a consequence of an increased synthesis of VLDLs (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 type 2 diabetes and healthy control subjects, glucose and fatty acid fluxes into the liver have been characterized (12–15). However, those techniques cannot discriminate between the effects of the liver versus those of the other splanchnic tissues. More recently, positron emission tomography (PET) was introduced to noninvasively assess hepatic substrate fluxes (16–18). To date, however, only a few studies have addressed effects of glucometabolic disorders on hepatic disposal of glucose and fatty acids in humans using PET (19–21).

Hepatic steatosis has also been associated with alterations of hepatic hemodynamics. Using noninvasive 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, noninvasive 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 type 2 diabetes.

The purpose of the current study was to measure hepatic perfusion and metabolism and to investigate the relationship with hepatic fat content in type 2 diabetic patients without diabetes-related complications and age-matched healthy male subjects.

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RESEARCH DESIGN AND METHODS

Fifty-nine type 2 diabetic patients and 18 healthy control subjects 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 control subjects were recruited by advertisements in local papers. Male type 2 diabetic patients, aged 45–65 years, without diabetes-related complications were eligible. Insulin pump dependence was not allowed. Whole-body insulin sensitivity (M/I value) was calculated as the mean plasma insulin level between 90 and 120 min from the start of the clamp procedure and then divided by the mean plasma insulin levels in the same time interval. A rise in 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 predicted ratio corresponds to the insulin 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 (~90 min after start of clamp) and after a second transmission scan, 11C]FDG was injected and a 60 min 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, nonesterified fatty acid, lactate, lipids, and insulin levels. In addition, 18O2 was measured during the [11C]palmitate scan (23,34).

PET data analysis. Emission data were corrected for physical decay of the respective tracers and for dead time, scatter, randoms, and photon attenuation. To generate myocardial time–activity curves, large regions (2 cm × 2 cm) of interest (ROIs) were defined in the right lobe of the liver on 4–5 consecutive frames with progressively increasing frame length. Blood samples were collected during all three scans at predefined time points to measure glucose, nonesterified fatty acid, lactate, lipids, and insulin levels. In addition, 18O2 was measured during the [11C]palmitate scan (23,34).

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Spectral analysis allows for assessment of (1) the number of tissue compartments identifiable in the data 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). ALT and AST were determined by automated colorimetric methods (Menarini Diagnostics, Amsterdam, The Netherlands). Levels of ALT and AST were calculated using Friedewald’s formula (reference values: 0–40 IU/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). Ultrasonic transcutaneous C-reactive protein (usCRP) was determined by ELISA (DSL, Webster, TX). 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 high-performance liquid chromatography (Menarini Diagnostics, Amsterdam). Plasma glucose was quantified using a hexokinase-based technique (Roche Diagnostics, Mannheim, Germany). A1C was determined by high-performance liquid chromatography (Bayer Diagnostics, Mijdrecht, The Netherlands). Plasma fatty acids were measured by an enzyme-linked immunoabsorbent assay (Bayer Diagnostics, Mijdrecht, The Netherlands).

Statistical analyses. Values are expressed as mean ± SE or as median (interquartile range) in case of skewed distribution. Nonnormally distributed data were log-transformed. Comparisons between control subjects, type 2 diabetes-low, and type 2 diabetes-high patients were performed using ANOVA, including the Bonferroni post hoc multiple comparisons test. Pearson and Spearman (where appropriate) univariate correlation coefficients were calculated, and linear regression was used to control for covariates. Statistical analysis was performed using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL). A two-tailed probability value < 0.05 was considered significant.

RESULTS

The 1H-MRS protocol was successfully completed in all participants. For technical reasons, four [15O]H2O, two [18F]FDG, and 12 [11C]palmitate scans in type 2 diabetic patients were not available for analysis, as well as one [15O]H2O and two [11C]palmitate scans in healthy control subjects.

Subject characteristics. Baseline characteristics of patients, categorized according to liver fat content, and control subjects are listed in Table 1. All groups were similar with respect to age, and both type 2 diabetes groups had comparable disease duration and medication use. As expected, anthropometric and hemodynamic parameters (which were all in the normal range) differed significantly between groups. Plasma lipid profiles and liver enzymes were different among 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. Type 2 diabetes-high patients had, compared with type 2 diabetes-low patients and control subjects, 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 vs. 572 ± 39 and 598 ± 52 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. Fig. 2 shows representative OSEM (summed) reconstructed PET images of the liver for [11C]palmitate, [18F]FDG, and 12 [11C]palmitate scans in type 2 diabetic patients with liver triglyceride content <8.6%. T2DM-low indicates type 2 diabetic patients with liver triglyceride content ≥8.6%. *indicates significant difference between controls and T2DM-low. †indicates significant difference between controls and T2DM-high. ‡indicates significant difference between T2DM groups. HbA1c, glycated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transerase.
**TABLE 2**

Metabolic characteristics in control and type 2 diabetic patients with low and high hepatic triglyceride content

| Metabolic characteristic (fasting state) | Control          | T2DM-low | T2DM-high | ANOVA       | P value |
|------------------------------------------|------------------|----------|-----------|-------------|---------|
| Plasma glucose, mmol/l                   | 5.2 (4.9–5.4)    | 8.3 (6.7–10.1)† | 8.0 (7.1–8.7)* | <0.001     |
| Plasma nonesterified fatty acids, umol/l| 470 (360–540)    | 450 (410–570)† | 500 (370–590)† | 0.624     |
| Plasma lactate, mmol/l                   | 0.8 (0.7–0.9)    | 1.1 (0.9–1.3)† | 1.2 (1.0–1.5)* † | <0.001     |
| Plasma insulin, pmol/l                   | 28 (19–33)       | 39 (28–62)† | 78 (62–99)* † | <0.001     |

| Metabolic characteristics (hyperinsulinemic state) | Control          | T2DM-low | T2DM-high | ANOVA       | P value |
|----------------------------------------------------|------------------|----------|-----------|-------------|---------|
| Plasma nonesterified fatty acids, umol/l           | 40 (20–48)       | 50 (30–85)† | 115 (70–173)* † | <0.001     |
| Plasma lactate, mmol/l                             | 1.1 (0.9–1.3)    | 1.0 (0.9–1.2)† | 1.1 (1.0–1.4)† | 0.560     |
| Plasma insulin, pmol/l                             | 511 ± 67         | 513 ± 23 | 643 ± 26)* † | <0.001     |
| M/I value, mg/(kg · min)/(pmol/l)                  | 1.13 (0.73–1.66) | 0.68 (0.46–1.0)† | 0.37 (0.17–0.45)* † | <0.001     |
| Insulin clearance rate, ml/min                     | 1,101 (1,017–1,270) | 1,029 (951–1,262) | 945 (816–1,053)* † | 0.003     |
| Post-hepatic insulin delivery rate, pmol/min       | 29 (21–40)       | 48 (30–77)† | 78 (56–97)* † | <0.001     |

Data are mean ± SE, median (interquartile range). T2DM-low = type 2 diabetic patients with liver triglyceride content ≤8.6%. T2DM-high = type 2 diabetic patients with liver triglyceride content >8.6%. *indicates significant difference between controls and T2DM-high. †indicates significant difference between controls and T2DM-low. ‡indicates significant difference between T2D groups. M/I value, whole-body insulin sensitivity; M value, whole-body M value adjusted for insulin during the steady state.

[18F]FDG, and fits on the data for the respective tracers. Type 2 diabetes-high patients had, compared with type 2 diabetes-low patients and control subjects, the lowest hepatic perfusion (0.647 ± 0.038 vs. 0.795 ± 0.042 and 0.850 ± 0.047 ml·min⁻¹, ANOVA, P = 0.004, Fig. 3A). Type 2 diabetes-high patients had, compared with type 2 diabetes-low patients and control subjects, the lowest insulin mediated HGU (20.4 ± 1.9 vs. 24.1 ± 2.1 and 30.7 ± 3.0 µmol·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 (Fig. 3C) was lower in type 2 diabetes-high patients compared with type 2 diabetes-low patients and control subjects, 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; Fig. 3A) and hepatic fatty acid influx rate constant (r = −0.335, P = 0.004; Fig. 3B), which both remained significant after correction for diabetic status, A1C, BMI, visceral fat content, plasma fatty acid, and lactate levels. Hepatic triglyceride content also correlated inversely with HGU (r = −0.329, P = 0.004; Fig. 3C), which remained signifi-

**FIG. 1.** Representative images of OSEM (summed) reconstructed PET images of the liver with [11C]palmitate (A) and [18F]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 [18O]H₂O concentration (C) in the liver (circles), with hepatic perfusion model fit (straight line). Patlak plots of [11C]palmitate (D) and [18F]FDG (E) data points, respectively. The blue dots in parts (D) and (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 (A) and (B), which is much higher for 11C-palmitate (fasting state) than for 18F-FDG (hyperinsulinemic state). (A high-quality digital representation of this figure is available in the online issue.)
FIG. 2. Hepatic perfusion (A), HGU (B), and hepatic fatty acid influx rate constant (C), in control subjects (●), type 2 diabetic patients with low liver triglyceride content (T2DM low) (■), and type 2 diabetic patients with high liver triglyceride content (T2DM high) (gray scale). $K_i$ = hepatic influx rate constant. $P$ values are from Bonferroni post hoc analysis. For $P$ ANOVA, see text.

Hepatic fat content, but not hepatic perfusion or hepatic fatty acid influx rate constant, correlated inversely with hepatic triglyceride content (r = −0.684, $P < 0.001$) and usCRP (r = 0.326, $P = 0.005$), insulin clearance rate (r = 0.459, $P < 0.001$), and visceral (r = 0.612, $P < 0.001$) and subcutaneous fat (r = 0.392, $P < 0.001$) volumes. The hepatic glucose influx rate constant correlated inversely with plasma fatty acid levels (r = −0.246, $P = 0.036$), A1C (r = −0.310, $P = 0.007$), and malondialdehyde (r = −0.434, $P < 0.001$).

In type 2 diabetic 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, $P = 0.057$).

FIG. 3. Correlations between hepatic triglyceride content % and hepatic perfusion (A), hepatic fatty acid influx rate constant (B), and HGU (C), in pooled analysis of type 2 diabetic patients and control subjects.

Hepatic fat content, but not hepatic perfusion or hepatic fatty acid influx rate constant, correlated inversely with M/I value (r = −0.657, $P < 0.001$) and usCRP (r = 0.375, $P = 0.005$), insulin clearance rate (r = −0.436, $P = 0.001$), and visceral (r = 0.540, $P < 0.001$) and subcutaneous fat (r = 0.375, $P = 0.003$) volumes. The hepatic glucose influx rate constant is inversely correlated with malondialdehyde (r = −0.380, $P = 0.004$) and borderline correlated with plasma fatty acids (r = −0.251, $P = 0.059$). None of these correlations were observed in control subjects alone.

DISCUSSION
Using MRS and PET in the same patients, the current study provides evidence for a potential modulating effect of hepatic fat content on hepatic physiology in type 2 diabetic patients. Reduced hepatic parenchymal perfusion, insulin-mediated HGU, and a borderline decrease in hepatic fatty acid influx rate constant were observed in type 2 diabetic
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, noninvasive measurements of hepatic perfusion using PET with the freely diffusible flow tracer $[^{15}O]$H$_2$O have been shown to provide reliable estimates of hepatic blood flow, when taking into account the dual input from hepatic artery and vena portae (27,28). In the current study, decreased hepatic parenchymal perfusion was observed in type 2 diabetic patients with increased liver triglyceride content but not in those type 2 diabetic patients with low liver triglyceride content, as compared with control subjects, 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 the degree of fat infiltration and both total hepatic blood flow and the hepatic parenchymal perfusion, with the biggest on the latter.

The mechanisms by which increased liver fat affects 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-κB have been shown to promote reactive oxygen species 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 type 2 diabetes 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 synthesis by endothelial cells and hence decreased nitric oxygen 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 current study no direct relationships were found between hepatic parenchymal perfusion and whole-body insulin sensitivity, oxidative stress, or ucCRP, the hepatic parenchymal 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 type 2 diabetes-high, but not type 2 diabetes-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 analog tracer $^{14}$R,S-$^{18}$F-fluoro-6-thia-heptadecanoic acid, Iozzo et al. found decreased fatty acid extraction in 10 fasting patients with impaired glucose tolerance compared with eight healthy control subjects (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 current study, during the $[^{11}$C{palm}ET scan, only venous sampling was performed and hence this relation could not be tested reliably.

In the current study, previous findings were confirmed, indicating that both type 2 diabetes 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 upregulation 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/heparine infusion increased plasma fatty acids and reduced splanchnic and peripheral glucose uptake in type 2 diabetic patients (50). Moreover, although the current 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 current study, we used $^1$H-MRS to measure hepatic triglyceride content. To that purpose, only three magnetic resonance 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 type 2 diabetes-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.

In conclusion, type 2 diabetic patients with high liver triglyceride content have a poorer metabolic profile than age-matched control subjects and type 2 diabetic patients with a liver triglyceride content in the normal range. In addition, type 2 diabetic 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.

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L.J.R. conceived and designed the study, analyzed and interpreted the data, and drafted and revised the manuscript. R.W.M. conceived and designed the study, analyzed and interpreted the data, and revised the manuscript. M.L. analyzed and interpreted the data, modeled PET data, provided technical assistance, and drafted and revised the manuscript. H.J.L., J.A.R., and A.R. conceived and designed the study and revised the manuscript. M.D. analyzed and interpreted the data, and revised the manuscript. R.W.M. conceived and designed the study, analyzed and interpreted the data, modeled PET data, and revised the manuscript. J.A.R. conceived and designed the study, modeled PET data, and revised the manuscript.

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