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Metabolism Clinical and Experimental 57 (2008) 1445 – 1451
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Inverse association between liver fat content and hepatic glucose uptake in patients with type 2 diabetes mellitus

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Received 16 January 2008; accepted 20 May 2008

Abstract

The objective of this research was to study (1) the mutual relationship between liver fat content (LFC) and hepatic glucose uptake (HGU) in patients with type 2 diabetes mellitus and (2) the relationship between changes in LFC and HGU uptake induced by rosiglitazone in these patients. Liver fat was measured with proton magnetic resonance spectroscopy and insulin-stimulated HGU with [^{18}F]-labeled 2-fluoro-2-deoxyglucose positron emission tomography in 54 patients with type 2 diabetes mellitus and 8 healthy subjects. Measurements were repeated in diabetic patients after a 16-week intervention period with rosiglitazone (n = 27) or placebo (n = 27). Patients with diabetes had lower HGU (24.5 ± 14.2 vs 35.6 ± 9.7 μmol/[kg min], $P < .01$) and higher LFC (10.9% ± 9.2% vs 2.5% ± 1.4%, $P < .001$) compared with healthy subjects. Liver fat was inversely associated with HGU ($r = -0.31$, $P < .05$), but more strongly with whole-body insulin sensitivity and adiponectin levels. Rosiglitazone treatment reduced liver fat by 24.8% ($P = .01$ vs placebo) and increased HGU by 29.2% ($P = .013$ vs placebo). This decrease in LFC was best explained by the increment in suppression of nonesterified fatty acid levels during hyperinsulinemia ($P < .001$) and improved glycemic control ($P = .034$), but not by changes in HGU. A significant inverse relationship between LFC and HGU was observed, but changes were not related. This suggests that the beneficial effects of rosiglitazone on liver metabolism are indirect and can be partly explained by increased suppression of nonesterified fatty acid levels, leading to reduced liver fat.

1. Introduction

Type 2 diabetes mellitus (T2DM) is generally characterized by insulin resistance. Although changes in liver glucose and fat metabolism in T2DM have been extensively studied, their individual role in T2DM remains unclear.

The pathology of nonalcoholic fatty liver disease (NAFLD) resembles that of alcohol-induced injury but occurs in patients who do not use alcohol. The prevalence of NAFLD is clearly increased in many obesity-related disorders. It has been shown that there is an increased relative risk for NALFD of 4.6 in obese persons with a body mass index (BMI) of a least 30 kg/m^2 [1]. Truncal obesity is an important risk factor for NAFLD, also in patients with a normal BMI [2]. Type 2 diabetes mellitus significantly increases the risk and severity of NAFLD regardless of BMI. A prevalence of NAFLD in T2DM of approximately 50% in the United States has been observed [3]. In patients with T2DM and severe obesity, 100% had at least mild steatosis, 50% had steatohepatitis, and 19% had cirrhosis [4]. Insulin resistance and hyperinsulinemia are associated with NAFLD also in subjects without T2DM [5]. Hypertriglyceridemia, but not hypercholesterolemia, is also an important risk factor for NAFLD [6]. High levels of circulating nonesterified fatty acids (NEFAs) have been suggested to cause an increase in liver triglyceride content [7]. Although liver fat content (LFC) is generally accepted to be higher in T2DM, we are not aware of previous reports on measurement of LFC in both T2DM patients and healthy volunteers within the same study.

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doi:10.1016/j.metabol.2008.05.015

Previously, only a few studies have evaluated hepatic glucose uptake (HGU). Studies using [^{18}F]-labeled 2-fluoro-2-deoxyglucose positron emission tomography ([^{18}F]FDG-PET) have shown that HGU may be stimulated by insulin [8]. Insulin-mediated liver glucose uptake is impaired in T2DM [9]. Rosiglitazone has been shown to increase HGU in patients with T2DM [10]. Increase in circulating NEFAs by intralipid infusion has been shown to lower HGU in healthy volunteers [11]. Whether HGU is related to the degree of LFC is unclear.

The main objective of our study was to investigate LFC and HGU and their mutual relationship in healthy subjects and in patients with T2DM. We hypothesized that LFC would be related to HGU. In addition, the aim was to evaluate whether the beneficial effects of rosiglitazone on LFC and HGU in T2DM patients are associated. To obtain our objectives, proton magnetic resonance spectroscopy (^{1}H MRS) and [^{18}F]FDG-PET were used. ^{1}H MRS is a noninvasive, highly sensitive application of a clinical MR imager to measure tissue fat content. [^{18}F]FDG-PET is a method of choice to measure liver glucose metabolism noninvasively and quantitatively.

2. Patients and methods

2.1. Subjects

Fifty-four patients with T2DM and 8 healthy subjects were included in the study (Table 1). Patients with T2DM were participating in another clinical trial with rosiglitazone therapy at our center [12]. Inclusion criteria for the study subjects were good to moderate glycemic control (hemoglobin A_{1c} [HbA_{1c}] <8.5%) and T2DM treated with diet or metformin and/or sulfonylurea. Exclusion criteria were alcohol or other drug abuse, clinical signs of heart failure, insulin therapy, and diabetes treated with thiazolidinediones. All patients were on stable medical therapy and had ischemic coronary heart disease. In addition, 8 healthy volunteers were studied. Written informed consent was obtained from all subjects after providing detailed information on the nature, purpose, and possible risks of the study. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland, and the study was conducted according to the guidelines of the Declaration of Helsinki.

2.2. Study design

Healthy subjects and T2DM patients who fulfilled the inclusion and exclusion criteria entered the study protocol. The ^{1}H MRS and PET examinations were performed on separate days. Patients with T2DM entered a 4-week run-in period before randomization and treatment either with rosiglitazone (4-8 mg/d) or placebo for a 16-week double-blinded trial. Characteristics of both rosiglitazone and placebo groups are displayed in Table 2. Patients with T2DM underwent ^{1}H MRS and [^{18}F]FDG-PET of the liver before and after the intervention.

2.3. ^{1}H MRS and magnetic resonance imaging studies

Patients were instructed to fast before the ^{1}H MRS examination. In addition to the ^{1}H MRS measurement of LFC, magnetic resonance imaging (MRI) was performed in the same examination for calculation of abdominal fat distribution. A 1.5-Tesla MR imager (Signa Horizon LX; GE Medical Systems, Milwaukee, WI) with the general-purpose flex surface coil and body coil was used for MRI and MRS. A coronal scout image of the abdominal area was obtained followed by transverse T1-weighted (T1W) dual-echo fast spoiled gradient echo (FSPGR) (out-in phase) imaging during breath hold for localization of the liver. A single voxel was positioned by an experienced radiologist in the liver parenchyma outside the area of the great vessels. Voxel dimensions (mean volume, 28.1 ± 6.9 cm^3) were adjusted to avoid visible vascular structures, taking into account the chemical shift artifact. Voxel location and size were recorded in each patient to ensure identical voxel placement before and after intervention. However, a recent multivoxel ^{1}H MRS liver study showed an intervoxel SD of 0.3% to 0.5% in healthy volunteers [13], implicating a negligible effect of small variations in voxel location on the final result. A point resolved spectroscopy (PRESS) ^{1}H MRS sequence (PROBE-SV, GE Medical Systems) was used with the following parameters: repetition time = 3000 milliseconds, echo time = 25 milliseconds, number of excitations = 8, and number of signals = 48, with a total duration of 2 minutes and 36 seconds. During this sequence, data were acquired during 2 breath hold intervals of 21 seconds. A typical location for the voxel is displayed in the T1W image with the corresponding spectrum (Fig. 1). In addition, a single T1W fast spin echo image was obtained at the level of the intervertebral disc L2-3 for analysis of abdominal adipose tissue masses as previously described by Abate et al [14]. Adipose tissue density of 0.9196 g/mL was used for converting measured volumes into weight.

2.4. Calculation of liver fat

The time domain fitting of the signal was performed using the Java Magnetic Resonance User Interface version 2001 data analysis package (http://www.mrui.uab.es/mrui). Fat signal amplitudes were calculated from the frames with water suppression using an advanced method for accurate, robust, and efficient spectral fitting (AMARES) [15]. The methylene and methyl peak amplitudes of the fat spectrum and amplitude of the water spectrum were corrected because of different T2 decay [16] and molar concentrations of ^{1}H nuclei in fat and water [17]. *Liver fat content* was defined as fat in relation to the total weight of liver tissue using the formula LFC (%) = (Sf)/(Sf + Sw/0.7), with Sf and Sw indicating areas of the fat and water peaks in the spectrum, respectively [16]. ^{1}H MRS findings of the liver have been validated in both animal and human studies [17,18].

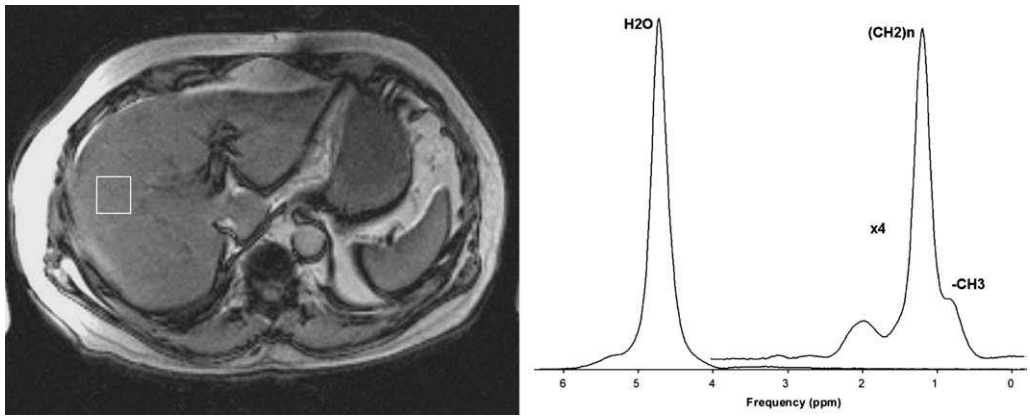

Fig. 1. Typical voxel location for ^{1}H MRS and the corresponding spectrum.

2.5. Positron emission tomography

Before the PET examination, the study subjects had fasted and refrained from all regular medications for 12 hours. Two catheters were inserted, both in the antecubital vein: one for blood sampling and the other for injection of [^{18}F]FDG and infusion of insulin and glucose. A heating pad was used to arterialize the venous blood in the arm for blood sampling. A euglycemic hyperinsulinemic clamp was used as previously described [19]. Insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was infused at a constant rate of 1 mU/(kg min); and euglycemia (5 mmol/l) was maintained by variable infusion of 20% glucose, depending on the plasma glucose concentration that was determined every 5 to 10 minutes. Hepatic glucose uptake was measured during hyperinsulinemic euglycemic clamp.

[^{18}F]FDG was produced as previously described by Hamacher et al [20] and injected intravenously over 1 minute (220-260 MBq) after 90 minutes of insulin stimulation. Scanning was performed using an ECAT 931/08-12 scanner (Siemens/CTI, Knoxville, TN) with the subjects in a supine position. Photon attenuation was corrected by a transmission scan of 5 minutes using a removable ^{68}Ge ring source. After [^{18}F]FDG injection, the liver was imaged during a 40-minute dynamic scan with frames of 8 × 15, 2 × 30, 2 × 120, 1 × 180, and 6 × 300 seconds, after which the femoral region was imaged during 5 × 240 seconds, as described previously [21]. During the scanning, blood samples were drawn to measure plasma and whole-blood [^{18}F]FDG radioactivity over time. All obtained data were corrected for tissue attenuation, dead time, and decay and reconstructed in a 128 × 128 matrix by a Bayesian iterative reconstruction algorithm [22]. Hepatic glucose uptake was quantitated as previously described [10,23]. A recent study has shown the validation for quantification of HGU in an animal model [24].

2.6. Biochemical analysis

Plasma glucose concentration during euglycemic hyperinsulinemic clamp was measured in duplicate by glucose oxidase method (Analox GM7 or GM9; Analox Instruments, London, United Kingdom). Other laboratory samples were sent to a central laboratory (Quest Diagnostics, London, United Kingdom). Standard methods and quality control were performed. Serum adiponectin level was measured by radioimmunoassay (human adiponectin radioimmunoassay kit; LINCO Research, St Charles, MO).

2.7. Statistical methods

The statistical analysis was performed with 8 healthy subjects and 54 T2DM patients. Data are reported as mean ± SD, and 2-tailed P values were used. One-way analysis of variance, and Kruskal-Wallis test in case of non-normally distributed variables, was used to compare LFC, HGU, glycemic control, and metabolic characteristics between the healthy subjects and the patients with T2DM, and then between the rosiglitazone and placebo groups. To compare the other variables between the treatment groups, unpaired t tests were used. Student paired t test was used to compare the values between baseline and week 16 in each group. For correlation analysis, Pearson correlation coefficients, or Spearman correlation coefficients in case of non-normally distributed variables, were calculated. Stepwise linear regression analysis was performed to determine the role of LFC in HGU values. A general linear model adjusting for BMI and age was used to evaluate the differences in LFC and HGU between patients with T2DM and healthy controls. A mixed model was used to assess possible effects of sex on the results. P value less than .05 was considered statistically significant. Statistical analyses were performed with SAS (Cary, NC) statistical analysis system 8.2.

3. Results

3.1. LFC and HGU in healthy subjects and patients with T2DM

Diabetic patients had poorer glycemic control compared with healthy subjects (Table 1). Whole-body insulin

Table 1
Study subjects characteristics

	Diabetic patients (n = 54)	Healthy subjects (n = 8)	P
Demography			
Sex (male/female)	38/16	8/0	
Age (y)	63.6 ± 7.5	54.0 ± 9.6	.002
Metabolic characteristics			
BMI (kg/m^2)	29.6 ± 4.1	26.4 ± 2.6	.037
HbA_{1c} (%)	7.2 ± 0.9	5.2 ± 0.3	<.0001
C-peptide (nmol/L)	0.84 ± 0.32	0.59 ± 0.14	.012
F-NEFA (mmol/L)	0.77 ± 0.3	0.24 ± 0.3	<.0001
Total cholesterol (mmol/L)	4.43 ± 0.77	5.68 ± 0.97	<.0001
Triglycerides (mmol/L)	1.8 ± 0.9	1.3 ± 0.5	.15
LDL cholesterol (mmol/L)	2.51 ± 0.68	3.85 ± 0.95	<.0001
HDL cholesterol (mmol/L)	1.12 ± 0.29	1.23 ± 0.21	.32
F-Insulin (pmol/L)	51.0 ± 30.0	38.3 ± 18.7	.25
ALAT (U/L)	25.6 ± 11.8	42.3 ± 28.6	.10
GT (U/L)	36.6 ± 35.13	49.4 ± 38	.14
Liver fat (%)	10.9 ± 9.2	2.5 ± 1.4	.0005
HGU (μmol/[kg·min])	24.5 ± 14.2	35.6 ± 9.7	.008
Whole-body glucose uptake (μmol/[kg·min])	11.9 ± 5.1	16.0 ± 4.1	.037
Subcutaneous fat mass (kg)	3.9 ± 1.4	3.1 ± 0.9	.12
Visceral fat mass (kg)	2.4 ± 0.9	1.6 ± 0.6	.019
Retroperitoneal fat mass (kg)	1.4 ± 0.6	1.0 ± 0.2	.002

LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; GT, γ-glutamyl transferase.

sensitivity (P = .012) was significantly lower in diabetic patients as compared with healthy subjects.

Liver fat content was significantly higher in patients with T2DM than in healthy subjects (P = .0005, Table 1). Hepatic glucose uptake was significantly decreased in diabetic patients compared with controls (P = .008). These differences in LFC (P = .012) but not in HGU (P = .11) remained significant when correcting for differences in age and BMI between patients with T2DM and controls.

3.2. Associations between LFC and HGU

Liver fat content was significantly and inversely associated with HGU in diabetic patients (r = −0.31, P = .025, Fig. 2A) but not significantly in the smaller group of healthy subjects (r = −0.6460, P = .08). In concert with that, LFC showed highly significant inverse correlations with the whole-body glucose uptake (r = −0.47, P = .0004, Fig. 2B) in diabetic patients but not in healthy subjects. The observed relationship between LFC and HGU was identical for both sexes (P = .53). Liver fat content was significantly correlated to visceral fat mass measured by MRI in patients with T2DM (r = 0.34, P = .013, Fig. 2C) and in healthy controls (r = 0.77, P = .02). Furthermore, LFC was significantly associated with retroperitoneal fat mass (r = 0.30, P = .032), BMI (r = 0.36, P = .008), triglyceride levels (r = 0.36, P = .009), NEFA levels during clamp (r = 0.45, P = .0008), adiponectin levels (r = −0.48, P = .0004), C-peptide levels (r = 0.42, P = .002), and age (r = −0.34, P = .015) in T2DM patients. Finally, LFC was significantly associated to alanine aminotransferase (ALAT) (r = 0.37, P = .007) and γ-glutamyl transferase (r = 0.29, P = .040) in patients with T2DM. By using stepwise linear regression with the model including all these parameters, LFC was best explained by

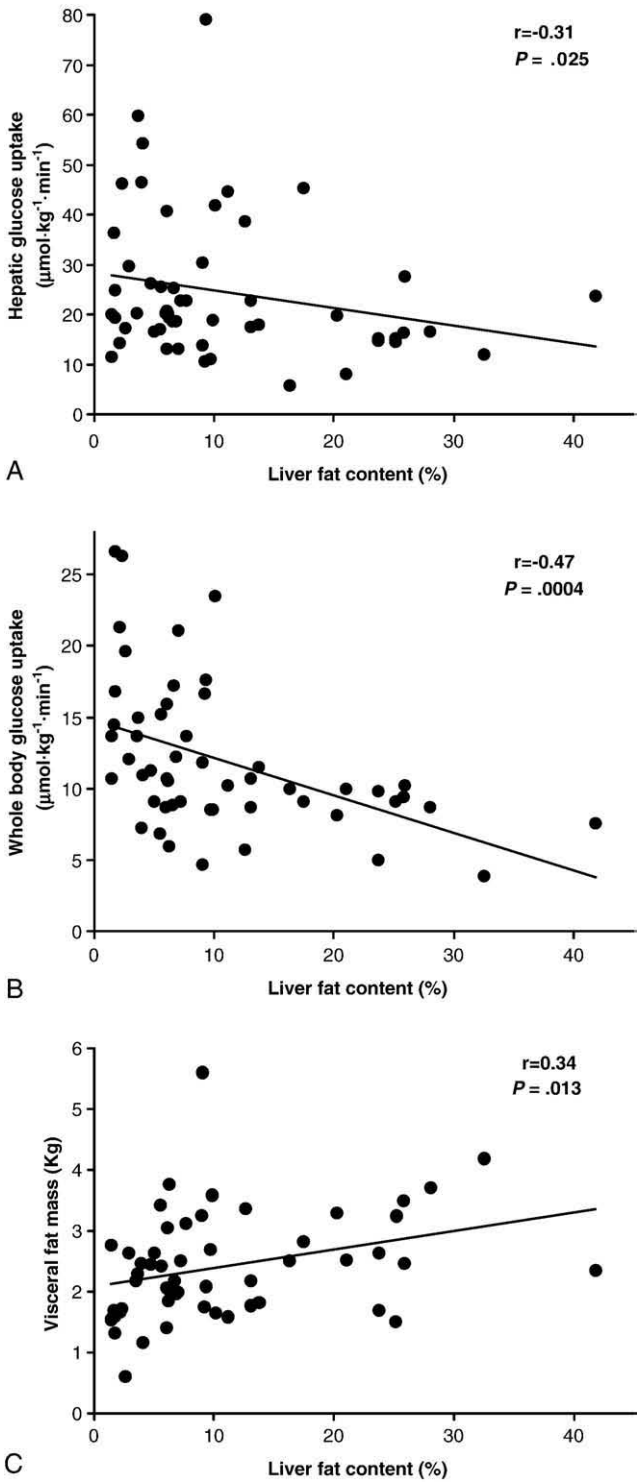

Fig. 2. Liver fat content was significantly correlated with hepatic insulin-stimulated glucose uptake (A), whole-body glucose uptake (B), and visceral fat mass (C) in patients with T2DM.

whole-body glucose uptake (M value) (P = .0009) and adiponectin levels (P = .016).

Hepatic glucose uptake was inversely associated with fasting C-peptide (r = −0.31 P = .026), fasting insulin level (r = −0.35, P = .01), subcutaneous fat mass (r = −0.33, P = .0140), and BMI (r = −0.27, P = .045) in T2DM patients. Using stepwise multiple regression analysis of significantly associated parameters, fasting insulin level was the strongest contributing factor to HGU in T2DM (P = .017).

3.3. Effects of rosiglitazone in T2DM patients

The groups were well matched for fasting plasma glucose, for C-peptide, and for insulin levels at the time of randomization (Table 2). Rosiglitazone significantly improved glycemic control compared with placebo. Effects of rosiglitazone on cholesterol were published previously by our group [25] and are therefore not assessed here. During hyperinsulinemia, steady-state plasma glucose concentrations were similar between the groups at baseline and at week 16. Serum NEFA concentrations (P = .04 vs baseline, P = .014 vs placebo) and insulin levels decreased during the clamp in the rosiglitazone group (P = .004 vs baseline, P = .006 vs placebo). The whole-body glucose uptake (P < .0001) increased in the rosiglitazone group as compared with the placebo group. Rosiglitazone increased HGU by 29.2% (P = .013 vs placebo, Table 1 and Fig. 3A). No changes occurred in the placebo group.

Rosiglitazone significantly reduced LFC by 24.8% (P = .010 vs placebo, Table 1 and Fig. 3B). Subcutaneous fat mass, visceral fat mass, and retroperitoneal fat mass did not change vs placebo. However, within the rosiglitazone group, a trend of increase in subcutaneous fat mass (P = .068) and decrease of visceral fat mass was observed (P = .175).

The overall response to treatment was identical for men and women concerning both the decrease in LFC (P = .81) and the increase in HGU (P = .83).

Stepwise multiple regression analysis, with the model including all significantly associated parameters, was used to explain the changes in LFC and HGU in the rosiglitazone group. The increase in suppression of NEFA levels during clamp (P = .0002) and increase in HbA_{1c} levels (P = .034) best explained the decrease in LFC. Improvement in HGU was best correlated with increase in HDL levels (P = .002).

4. Discussion

The present study is the first to show a significant inverse relationship between LFC and hepatic glucose metabolism. Liver fat content was significantly increased and HGU was decreased in T2DM patients compared with healthy subjects, suggesting impaired tissue-specific metabolism and insulin resistance associated with tissue steatosis. The strong association of LFC to whole-body glucose uptake (P =

Table 2
Metabolic data of patients with T2DM and effects of rosiglitazone

	Baseline		After 16 wk		P[a]
	Placebo	Rosiglitazone	Placebo	Rosiglitazone	
At fast					
Plasma glucose (mmol/L)	7.7 ± 1.7	7.3 ± 2.0	8.1 ± 2.3	6.0 ± 1.1	<.0001
HbA_{1c} (%)	7.1 ± 0.9	7.3 ± 0.9	7.3 ± 1.0	6.9 ± 0.6	<.0001
Serum insulin (pmol/L)	53 ± 26	49 ± 34	56 ± 36	34 ± 17	.003
Serum NEFAs (mmol/L)	0.81 ± 0.3	0.73 ± 0.2	0.80 ± 0.3	0.67 ± 0.2	NS
Total cholesterol (mmol/L)	4.68 ± 0.8	4.19 ± 0.67	4.70 ± 0.88	4.70 ± 0.88	.014
Triglycerides (mmol/L)	1.9 ± 1.0	1.7 ± 0.8	1.8 ± 1.0	1.7 ± 1.2	NS
LDL cholesterol (mmol/L)	2.67 ± 0.73	2.35 ± 0.61	2.68 ± 0.67	2.61 ± 1.0	.015
HDL cholesterol (mmol/L)	1.17 ± 0.35	1.07 ± 0.2	1.18 ± 0.35	1.14 ± 0.25	.051
Serum C-peptide (nmol/L)	0.87 ± 0.3	0.82 ± 0.3	0.85 ± 0.4	0.69 ± 0.2	NS
Adiponectin (μg/mL)	7.2 ± 4.4	7.2 ± 2.6	7.4 ± 3.9	19.0 ± 7.9	<.0001
During hyperinsulinemia					
Plasma glucose (mmol/L)	5.24 ± 0.53	5.28 ± 0.42	5.36 ± 0.75	5.23 ± 0.36	NS
Serum insulin (pmol/L)	439 ± 88	440 ± 78	441 ± 76	405 ± 66	.006
Serum NEFAs (mmol/L)	0.16 ± 0.08	0.14 ± 0.06	0.15 ± 0.06	0.10 ± 0.06	.014
Whole-body glucose uptake (μmol/[kg·min])	11.5 ± 4.2	12.3 ± 6.0	12.0 ± 5.5	17.8 ± 6.8	<.0001
HGU (μmol/[kg·min])	25.2 ± 11.2	23.8 ± 16.9	25.4 ± 11.2	33.6 ± 21.3	.013
^1H *MRS and MRI*					
Liver fat (%)	10.1 ± 8.0	11.7 ± 10.4	9.8 ± 8.2	8.8 ± 10.2	.010
Subcutaneous fat mass (kg)	3.92 ± 1.4	3.87 ± 1.4	3.94 ± 1.3	4.12 ± 1.4	NS
Visceral fat mass (kg)	2.42 ± 0.8	2.42 ± 1.0	2.46 ± 0.8	2.32 ± 1.0	NS
Retroperitoneal fat mass (kg)	1.39 ± 0.5	1.40 ± 0.7	1.39 ± 0.5	1.44 ± 0.7	NS

NS indicates not significant.

[a] Change in rosiglitazone group vs change in placebo group.

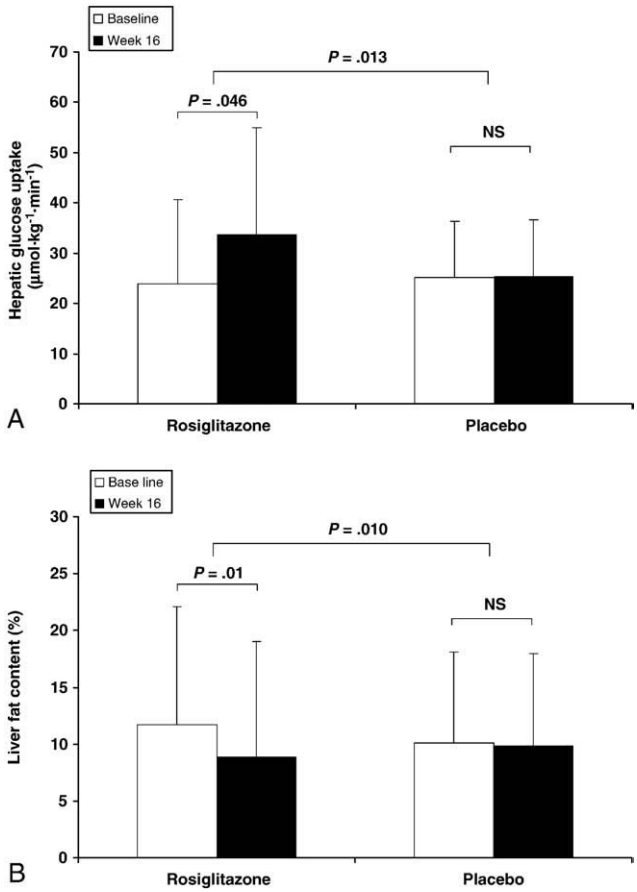

Fig. 3. The effect of rosiglitazone on HGU (A) and LFC (B).

.0004) observed in our study supports the important role of LFC in insulin resistance.

Although rosiglitazone treatment had beneficial effects on both liver steatosis and HGU, the individual changes were variable; and no relationship of the changes was observed. However, changes in clamp NEFA levels were strongly associated with changes in LFC. Decrease of NEFA levels indicates overall improvement of suppression of lipolysis. This might result in decreased portal NEFA load and thereby reduce LFC in patients treated with rosiglitazone. Although HGU has been shown to be dependent on plasma NEFA level [11], suppression of lipolysis was not significantly associated with changes in HGU in the rosiglitazone group. This suggests that the increase in HGU by rosiglitazone action is only partly explained by reduced NEFA levels. Additional direct effects of rosiglitazone on the liver possibly play an important role. Peroxisome proliferator–activated receptor–γ agonists have been shown to increase glycogen storage and decrease gluconeogenesis through direct effects on the hepatocyte in vitro [26,27].

Previous studies have shown that thiazolidinediones promote redistribution of ectopic fat to subcutaneous fat depots. In the present study with rosiglitazone, only trends of increase in subcutaneous fat mass (P = .068) and decrease of visceral fat mass were observed (P = .175). This is in concert with previously observed borderline changes in studies by us [10] and others [28] assessing the effects of rosiglitazone on abdominal visceral and subcutaneous fat masses by MRI. The correlation between LFC and visceral fat mass in the current study supports the essential role of increased visceral fat mass in the development of NALFD in T2DM.

We have previously shown that liver steatosis coexists with myocardial insulin resistance and coronary dysfunction in patients with T2DM [29]. Liver fat content as assessed by ^{1}H MRS is presumably a very sensitive and early indirect marker of metabolic dysregulation, for even a small increase in LFC was associated with changes in HGU and especially whole-body glucose uptake. Based on the association of LFC to ALAT shown in this study, increased LFC seems to be more a marker of inflammatory state of the liver than a factor impairing overall functionality. Patients with T2DM in this study had macrovascular complications. By taking into account the long preclinical period before diagnosis in T2DM, some degree of complications is present even in early-onset T2DM patients. Therefore, it is likely that the present findings apply to the general population of T2DM patients.

In conclusion, this study shows that increased LFC is associated with decreased hepatic insulin-stimulated glucose uptake in patients with T2DM. Rosiglitazone therapy simultaneously decreases LFC and improves HGU, but individual changes are not directly related. This suggests that the beneficial effects of rosiglitazone on liver metabolism can be partly explained by improved suppression of lipolysis, leading to reduced LFC, in addition to more direct drug effects. Larger studies need to be conducted to understand the role of LFC in insulin resistance and pathways by which both LFC and HGU are regulated and modulated by rosiglitazone to optimize treatment in patients with T2DM.

Acknowledgment

The Magnetic Resonance User Interface software package was kindly provided by the participants of the EU Network programs: Human Capital and Mobility, CHRX-CT94-0432, and Training and Mobility of Researchers, ERB-FMRX-CT970160. This research has been supported by a Marie Curie Fellowship of the European Community program QOL 1.1.1 under contract number QKGA-1999-51330. The authors would like to thank Irina Lisinen for her help with statistical analysis. We thank the staff of Turku PET Centre for their excellent technical assistance.

This work is part of the project Hepatic and Adipose Tissue and Functions in the Metabolic Syndrome (HEPADIP, http://www.hepadip.org/), which is supported by the European Commission as an Integrated Project under the Sixth Framework Programme (contract LSHMCT-2005-018734). The study was conducted within the Centre of Excellence in Molecular Imaging in Cardiovascular and Metabolic

Research and was supported by the Academy of Finland, University of Turku, Turku University Hospital, and Abo Academy. This study was further supported by a grant of GlaxoSmithkline, the Turku University Hospital (EVO), the Finnish Diabetes Foundation (to PI and RB), and the Ella and Georg Ehrnrooth Foundation (to RB).

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