Pioglitazone Reduces Hepatic Fat Content and Augments Splanchnic Glucose Uptake in Patients With Type 2 Diabetes

Mandeep Bajaj, Swangjit Suraamornkul, Thongchai Pratipanawatr, Lou J. Hardies, Wilailak Pratipanawatr, Leonard Glass, Eugenio Cersosimo, Yoshinori Miyazaki, and Ralph A. DeFronzo

The effect of pioglitazone on splanchnic glucose uptake (SGU), endogenous glucose production (EGP), and hepatic fat content was studied in 14 type 2 diabetic patients (age 50 ± 2 years, BMI 29.4 ± 1.1 kg/m^2, HbA_{1c} 7.8 ± 0.4%). Hepatic fat content (magnetic resonance spectroscopy) and SGU (oral glucose load–insulin clamp technique) were quantitated before and after pioglitazone (45 mg/day) therapy for 16 weeks. Subjects received a 7-h euglycemic insulin (100 mU · m^{-2} · min^{-1}) clamp, and a 75-g oral glucose load was ingested 3 h after starting the insulin clamp. Following glucose ingestion, the steady-state glucose infusion rate during the insulin clamp was decreased appropriately to maintain euglycemia. SGU was calculated by subtracting the integrated decrease in glucose infusion rate during the 4 h after glucose ingestion from the ingested glucose load. 3-[^{3}H]glucose was infused during the initial 3 h of the insulin clamp to determine rates of EGP and glucose disappearance (R_d). Pioglitazone reduced fasting plasma glucose (10.0 ± 0.7 to 7.5 ± 0.6 mmol/l, $P < 0.001$) and HbA_{1c} (7.8 ± 0.4 to 6.7 ± 0.3%, $P < 0.001$) despite increased body weight (83 ± 3 to 86 ± 3 kg, $P < 0.001$). During the 3-h insulin clamp period before glucose ingestion, pioglitazone improved R_d (6.9 ± 0.5 vs. 5.2 ± 0.5 mg · kg^{-1} · min^{-1}, $P < 0.001$) and insulin-mediated suppression of EGP (0.21 ± 0.04 to 0.06 ± 0.02 mg · kg^{-1} · min^{-1}, $P < 0.01$). Following pioglitazone treatment, hepatic fat content decreased from 19.6 ± 3.6 to 10.4 ± 2.1%, ($P < 0.005$), and SGU increased from 33.0 ± 2.8 to 46.2 ± 5.1% ($P < 0.005$). Pioglitazone treatment in type 2 diabetes *1*) decreases hepatic fat content and improves insulin-mediated suppression of EGP and *2*) augments splanchnic and peripheral tissue glucose uptake. Improved splanchnic/peripheral glucose uptake and enhanced suppression of EGP contribute to the improvement in glycemic control in patients with type 2 diabetes. *Diabetes* 52:1364–1370, 2003

From the Diabetes Division, Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas.

Address correspondence and reprint requests to Mandeep Bajaj, Assistant Professor, Diabetes Division, Department of Medicine, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284-7886. E-mail: mandeepbajaj@hotmail.com.

Received for publication 26 July 2002 and accepted in revised form 27 February 2003.

EGP, endogenous glucose production; FFA, free fatty acid; FPG, fasting plasma glucose; MRS, magnetic resonance spectra; PPARγ, peroxisome proliferator–activator receptor-γ; PRESS, Point Resolved Spectroscopy Sequence; SGU, splanchnic glucose uptake.

The splanchnic tissues play a pivotal role in the maintenance of normal glucose homeostasis (1). Hyperglycemia, plasma free fatty acid (FFA) concentration, and route of glucose administration all exert independent effects on splanchnic glucose uptake (SGU). When glucose is administered intravenously, the resultant hyperglycemia enhances SGU in proportion to the increase in plasma glucose concentration such that the splanchnic glucose clearance remains unchanged (2,3). This mass-action effect of hyperglycemia to augment SGU is dependent upon maintained portal insulin levels (2–5,8). Insulin per se does not increase SGU (2,5). Studies by DeFronzo and colleagues (3,5) in humans and by Cherrington and colleagues (6,7) in dogs have shown that the gastrointestinal/portal route of glucose administration has a specific enhancing effect on SGU. Thus, following glucose ingestion, the fractional, as well as absolute rate of glucose uptake by the splanchnic tissues is significantly greater than the combined effects of hyperinsulinemia plus hyperglycemia created by intravenous glucose/insulin administration (5,6). In type 2 diabetic individuals, a decrease in SGU has been demonstrated following ingestion of an oral glucose load (3,9–11).

Recent studies have demonstrated that the plasma FFA concentration also plays an important role in the regulation of SGU. In nondiabetic humans (12,13), an acute elevation in the plasma FFA concentration causes peripheral (muscle) insulin resistance and a concomitant increase (12) or a tendency toward an increase (13) in splanchnic (hepatic) glucose uptake following ingestion/infusion of a glucose load. In contrast, in type 2 diabetic subjects, increased plasma FFA levels induce peripheral insulin resistance but fail to augment SGU following an oral glucose load (14). To the contrary, SGU is reduced in response to the elevated plasma FFA concentration (14). This inhibitory effect of increased plasma FFA levels on SGU may, in part, account for the impairment on splanchnic (hepatic) glucose uptake following glucose ingestion. It is well documented that disturbances in FFA metabolism are a characteristic feature of type 2 diabetic individuals (1,15–17), who manifest day-long increased plasma FFA levels (17) and increased rates of lipolysis (1,15–17).

Recent studies have suggested that increased hepatic fat content is a strong predictor of hepatic insulin resistance

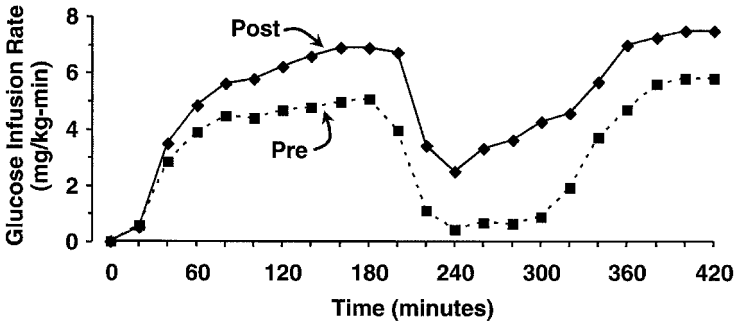

FIG. 1. Glucose infusion rate during the combined oral glucose load–insulin clamp (100 mU · m^{-2} · min^{-1}) before (pre) and after (post) pioglitazone treatment.

in type 2 diabetic patients (18–21). The mechanisms responsible for the increase in hepatic fat content are unclear. It has been suggested that fatty liver results from accelerated fatty acid mobilization from expanded visceral fat stores and their deposition in the liver (22). A decrease in hepatic fatty acid oxidation has been suggested as the cause of the increased hepatic fat content (23). Alternatively, the increased hepatic fat content simply could reflect an excessive intake of dietary fat. Whatever the mechanism, the metabolic consequences of the increase in hepatic fat content on SGU have not been studied in patients with type 2 diabetes.

The thiazolidinediones have become widely used to treat patients with type 2 diabetes. These drugs work by binding to peroxisome proliferator–activator receptor-γ (PPARγ), which is primarily located on adipocytes (24). In type 2 diabetic patients, thiazolidinedione therapy is associated with a reduction in circulating plasma FFA levels and FFA turnover (25), a shift in fat distribution from visceral to subcutaneous fat storage depots (26–28), a decrease in hepatic fat content (29), and an improvement in peripheral insulin sensitivity (29). However, no previous study has examined whether the decrease in hepatic fat content and/or plasma FFA concentration is related to improved SGU following thiazolidinedione treatment in patients with type 2 diabetes.

The current study was designed to determine the effect of pioglitazone therapy on hepatic fat content and plasma FFA concentration, SGU following glucose ingestion, and hepatic and peripheral tissue sensitivity to insulin in patients with type 2 diabetes. Hepatic fat content was determined using proton spectroscopy. To quantitate SGU, we used a combined euglycemic insulin clamp–oral glucose load technique developed in our laboratory (3,5) and subsequently modified by Ludvik et al. (30).

RESEARCH DESIGN AND METHODS

Subjects. Fourteen type 2 diabetic patients (9 men, 5 women; age 50 ± 2 years; 11 Mexican-American, 1 Caucasian, 1 African-American, 1 Asian-American; duration of diabetes 4 ± 1 years) participated in the study. Four subjects were taking a stable dose of sulfonylurea drugs for at least 3 months before study, and 10 subjects were treated with diet alone. Patients who had received insulin, metformin, or another thiazolidinedione in the previous 3 months were excluded. Entry criteria included age from 30 to 70 years, stable body weight for at least 3 months before the study, and fasting plasma glucose (FPG) concentration between 7.0 and 14.5 mmol/l. All patients were in good general health, without evidence of cardiac, hepatic, renal, or other chronic diseases as determined by history, physical examination, screening blood tests, and urinalysis. No subjects participated in any heavy exercise, and no subjects were taking any medications known to affect glucose metabolism. All subjects gave signed voluntary informed consent before participation. The Institutional Review Board of the University of Texas Health Science Center at San Antonio approved the protocol.

Study design. Three weeks before study, all subjects met with a dietitian and were instructed to consume a weight-maintaining diet containing 50% carbohydrate, 30% fat, and 20% protein. During the week before the start of pioglitazone treatment, all subjects received baseline measurement of FPG, FFA, and insulin (mean of three values drawn at 15-min intervals). At the same time, blood samples were taken for liver function tests, fasting plasma lipids, and HbA_{1c}; liver fat content was measured using proton spectroscopy; and a euglycemic insulin clamp in combination with a 75-g oral glucose load was administered to quantitate SGU. All studies were performed at 0800 h, following a 10- to 12-h overnight fast. Sulfonylurea-treated subjects discontinued their medication 48 h before the study.

Following completion of these studies, subjects were started on pioglitazone, 45 mg/day for 16 weeks. During the pioglitazone treatment period, subjects returned to the Clinical Research Center every 2 weeks at 0800 h following an overnight fast for measurement of FPG concentration, body weight, and blood pressure. Fasting plasma lipids (total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol) were measured monthly. HbA_{1c} was measured twice during the last week of pioglitazone treatment. On each visit, dietary adherence was reinforced. After 16 weeks of pioglitazone treatment, all subjects underwent a repeat oral glucose load–insulin clamp study to quantitate SGU and measurement of hepatic fat content by proton spectroscopy.

Oral glucose load–insulin clamp. Subjects were admitted to the General Clinical Research Center at 1800 h on the evening before the study and ate a standard weight-maintaining meal (55% carbohydrate, 30% fat, and 15% protein) between 1830 and 1900 h. After 2000 h, subjects refrained from eating or drinking anything except water. At 2200 h, a catheter was placed in the antecubital vein, and a variable low dose insulin infusion (8–12 mU · m^{-2} · min^{-1}) was initiated to reduce the plasma glucose concentration to ~5.6 mmol/l and maintain it there.

At 0800 h on the following day, a second catheter was inserted retrogradely into a vein on the dorsum of the hand for blood sampling, and the hand was placed in a heated box (60°C) for the duration of the study. A euglycemic insulin (100 mU · m^{-2} · min^{-1}) clamp was begun and continued for 7 h. Arterialized blood samples were collected every 5 min for plasma glucose determination, and a 20% glucose infusion was adjusted to maintain the plasma glucose concentration at ~5.6 mmol/l (31). During the first 180 min of the euglycemic insulin clamp, a primed (25 μCi) continuous (0.25 μCi/min) infusion of 3-[^{3}H]glucose was given to measure endogenous glucose production (EGP). The tritiated glucose infusion was discontinued after 180 min, when the glucose load was ingested. Insulin, glucose, and 3-[^{3}H]glucose were infused via the antecubital vein. Plasma samples for determination of plasma insulin concentration were obtained every 15–30 min throughout the study. Plasma samples for the determination of 3-[^{3}H]glucose specific activity were obtained every 5–10 min during the 150–180 min period of the euglycemic insulin clamp. During the 150–180 min period of the insulin clamp, the exogenous glucose infusion rate required to maintain euglycemia was constant (Fig. 1). At 3 h after starting the insulin clamp (1100 h), subjects ingested 75 g of glucose over a 5-min period. As the oral glucose was absorbed, the exogenous intravenous glucose infusion rate was reduced appropriately to maintain euglycemia (Fig. 1). After glucose ingestion, the plasma glucose concentration increased slightly (by ~1 mmol/l) during the 180–300 min time period in the pretreatment studies (Fig. 2), even though the exogenous glucose infusion rate was reduced to zero. Following pioglitazone treatment, the plasma glucose concentrations were matched for this increase in each individual patient. Within 3–3.5 h after glucose ingestion, the glucose infusion rate returned to or exceeded the rate at 180 min, indicating complete absorption of the oral glucose load (Fig. 1).

Liver fat content (proton magnetic resonance spectroscopy). Localized ^{1}H nuclear magnetic resonance spectra (MRS) of the liver were acquired on a 1.9 T MRI scanner (Prestige Elscint, Elscint, Haifa, Israel), using a standard

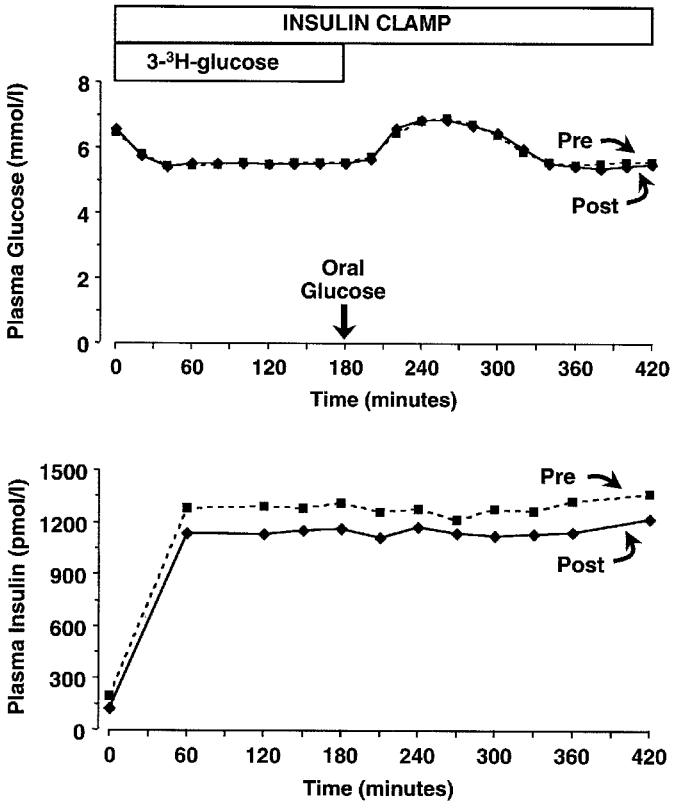

FIG. 2. Plasma glucose and insulin concentrations during the oral glucose load–insulin clamp before (pre) and after (post) pioglitazone treatment.

body coil in transmitter and receiver mode. An initial T1-weighted spin-echo anatomical magnetic resonance scan for liver MRS localization was performed with the following parameters: repetition time (TR)/echo time (TE)/θ = 130 ms/15 ms/160°; slice thickness = 7 mm; field of view = 44 cm × 45 cm; number of excitations = 1; and image matrix = 100 × 256. The slice with the largest gross dimensions of the liver was chosen for the MRS study. MRS for water and fat quantification were accomplished by using a Point Resolved Spectroscopy Sequence (PRESS) (32). The imaging parameters for PRESS sequence were as follows: TR/TE/θ = 1,500 ms/54 ms/90°; number of averages = 2; and data points = 512. A 3 cm × 3 cm × 3 cm volume (voxel) was selected in the left, right anterior, and right posterior hepatic lobes for scanning to provide a more generalized distribution of fat within the liver. During the measurements, the subject lay supine within the bore of the magnet. The total scan time was ~60 min. During the MRS examinations, identical areas of the liver were scanned in the pre- and post-treatment MRS studies of the same subject by the use of anatomical landmark visualizing images.

After line broadening and phase and baseline correction, the peak area of the water (S_w) at 4.77 ppm and fat resonance (S_f) at 1.4 ppm were measured. Quantification of the fat content was done by comparing the area of the fat resonance with that of the unsuppressed water. Spectroscopic data were processed using the Elscint operating system software. Hepatic fat percentage was calculated by dividing 100 times S_f by the sum of S_f and S_w. This technique is highly reproducible, with a CV of <2% when the same subjects were studied on 8 separate days. Hepatic fat content determined by the MRS technique is strongly correlated (r = 0.89) with hepatic fat content determined by histological techniques in humans undergoing liver biopsies, although the absolute values obtained from the two methods are not identical (33).

Analytical determinations. Plasma glucose concentration was measured by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin concentration was measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). Tritiated glucose specific activity was determined on deproteinized barium/zinc plasma samples as previously described (15). Plasma FFA concentration was determined by an enzymatic colorimetric quantification method (Wako Chemicals, Nuess, Germany).

Calculations. During the euglycemic insulin clamp before the ingestion of glucose (0–180 min), the rate of total body glucose appearance (R_a) was calculated using Steele's equation (34) and a distribution volume of 250 ml/kg. EGP was calculated by subtracting the exogenous glucose infusion rate from the tracer-derived measure of R_a. The rate of insulin-mediated total body glucose disposal (R_d) was determined by adding the rate of residual EGP to the exogenous glucose infusion rate. The tritiated glucose infusion was discontinued at 180 min, and EGP was not determined during the 180–420 min time period after the ingestion of glucose during this study.

Splanchnic glucose uptake was calculated as follows: the glucose infusion rate after oral glucose ingestion was subtracted from the reference glucose infusion rate to obtain the decrement in the exogenous glucose infusion rate. The reference glucose infusion rate was calculated as the mean of the glucose infusion rate during the 150–180 min time period (before glucose ingestion) and the 380–420 min time period. The integrated decrement in the exogenous glucose infusion rate after glucose ingestion was multiplied by the subject's body weight and by the time interval to calculate the amount of glucose escaping the splanchnic bed. The amount of glucose escaping the splanchnic bed was subtracted from the oral glucose load (75 g) to calculate the SGU. Previous studies (9,30,35) have shown that glucose absorption from the gastrointestinal tract after glucose ingestion is complete within 3–3.5 h, and this was confirmed in the present study by the sharp rise in the exogenous glucose infusion rate in all subjects to or above the pre–oral glucose load rate (150–180 min) by 380 min. This preceding calculation assumes that residual EGP during the combined oral glucose load–100 mU · m^{-2} · min^{-1} insulin clamp is negligible, which was documented with tritiated glucose during the insulin clamp before glucose ingestion. The combination of pharmacological hyperinsulinemia and portal hyperglycemia following glucose ingestion ensures the complete suppression of hepatic glucose production. Under these conditions, the net splanchnic glucose balance and SGU are synonymous.

Statistical analysis. Statistical calculations were performed with StatView for Windows, version 5.0 (SAS Institute, Cary, NC). Values before and after treatment were compared using the paired t test. Linear or logarithmic (for nonlinearly distributed data) regression analysis was used to examine the relationships between hepatic insulin sensitivity and SGU versus hepatic fat content. Multivariate analysis was performed where appropriate to examine the impact of simultaneous changes in experimental parameters on SGU. Data are presented as mean ± SE. A P value <0.05 was considered to be statistically significant.

TABLE 1
Anthropometric and laboratory measurements before and after pioglitazone treatment for 16 weeks

	Before	After	P value
Body weight (kg)	83.0 ± 3.0	86.0 ± 3.0	<0.001
BMI (kg/m^2)	29.4 ± 1.1	30.4 ± 1.1	<0.001
HbA_{1c} (%)	7.8 ± 0.4	6.7 ± 0.3	<0.001
Fasting plasma glucose (mmol/l)	10.0 ± 0.7	7.5 ± 0.6	<0.001
Fasting plasma insulin (pmol/l)	66 ± 12	36 ± 6	<0.01
Fasting plasma FFA (μmol/l)	699 ± 57	571 ± 55	<0.01
Total cholesterol (mg/dl)	203 ± 9	195 ± 8	
LDL cholesterol (mg/dl)	125 ± 8	123 ± 7	
HDL cholesterol (mg/dl)	45 ± 3	47 ± 4	
Triglycerides (mg/dl)	163 ± 23	124 ± 17	0.02
AST (IU/l)	27 ± 2	22 ± 1	0.01
ALT (IU/l)	28 ± 3	22 ± 2	0.02

Data are mean ± SE. AST, aspartate transaminase (normal range, 13–47 IU/l); ALT, alanine transaminase (normal range, 5–40 IU/l).

RESULTS

Metabolic parameters. Metabolic parameters are shown in Table 1. After 16 weeks of pioglitazone treatment, the FPG concentration decreased significantly, from 10.0 to 7.5 mmol/l, and the HbA_{1c} declined from 7.8 to 6.7% (P < 0.001), despite a 45% decline in the fasting plasma insulin concentration. Fasting plasma triglyceride (P = 0.02) and fasting plasma FFA (P < 0.01) concentrations decreased significantly following pioglitazone treatment. Total cholesterol, HDL cholesterol, and LDL cholesterol did not change significantly. Significant decreases in serum aspartate transaminase and alanine transaminase levels were observed following pioglitazone treatment.

Oral glucose load–insulin clamp: plasma glucose, insulin, and FFA concentrations. The plasma glucose concentrations following the overnight insulin infusion were similar during the oral glucose load–insulin clamp studies before and after pioglitazone treatment (6.4 ± 0.2 vs. 6.5 ± 0.1 mmol/l). During the initial 3 h of the euglycemic insulin clamp, the steady-state plasma glucose concentrations were similar before and after pioglitazone (5.6 ± 0.1 vs. 5.6 ± 0.1 mmol/l). After glucose ingestion, the plasma glucose concentrations were similar before and after pioglitazone (Fig. 2). From 180 to 300 min, there was a small rise in plasma glucose concentration that was similar in the pre- and postpioglitazone studies (Δ = 0.9 ± 0.2 vs. Δ = 0.9 ± 0.2 mmol/l). The plasma glucose concentration returned to ~5.6 mmol/l and remained constant at this level between 300 and 420 min (5.6 ± 0.1 vs. 5.6 ± 0.1 mmol/l) before and after pioglitazone therapy (Fig. 2).

The plasma insulin concentrations (Fig. 2) did not differ significantly during the 180-min euglycemic insulin clamp (1,294 ± 104 vs. 1,154 ± 98 pmol/l) or during the oral glucose load–insulin clamp (180–420 min) (Fig. 2) before and after pioglitazone treatment. During the 180–300 min time period, when the plasma glucose concentration rose slightly, there was no increase in the plasma insulin concentration.

During the 150–180 min period of the insulin clamp, suppression of plasma FFA concentration was significantly enhanced after pioglitazone treatment (121 ± 17 vs. 169 ± 20 μmol/l, $P < 0.05$).

Glucose metabolism during oral glucose load–insulin clamp. The time course of the exogenous intravenous glucose infusion rate is shown in Fig. 1. During the insulin clamp studies performed before and after pioglitazone therapy, the mean glucose infusion rate increased steadily during the initial 150 min and reached a plateau from 150 to 180 min. The glucose infusion rate was significantly greater during the 150–180 min time period of the euglycemic insulin clamp (6.9 ± 0.5 vs. 5.0 ± 0.5 mg · kg^{-1} · min^{-1}, $P < 0.001$) after pioglitazone treatment. Following glucose ingestion, there was an abrupt decline in the glucose infusion rate required to maintain euglycemia (Fig. 1). By 380 min, the glucose infusion rate returned to the pre–oral glucose load value in all subjects, indicating complete absorption of the oral glucose load. During the 180–420 min period of the oral glucose load–insulin clamp, the glucose infusion rate was significantly increased following pioglitazone treatment (5.3 ± 0.5 vs. 2.9 ± 0.5 mg · kg^{-1} · min^{-1}) ($P < 0.001$).

Insulin-mediated suppression of EGP, determined during the 150–180 min period of the euglycemic insulin clamp, was significantly enhanced after pioglitazone treatment (0.06 ± 0.02 vs. 0.21 ± 0.04 mg · kg^{-1} · min^{-1}, respectively; $P < 0.01$). Following pioglitazone treatment, the whole body glucose disposal rate (R_d) was significantly increased from 150–180 min (6.9 ± 0.5 vs. 5.2 ± 0.5 mg · kg^{-1} · min^{-1}, $P < 0.001$).

SGU. SGU during the oral glucose load–insulin clamp was significantly increased after pioglitazone treatment (34.7 ± 3.8 vs. 24.7 ± 2.1 g, $P < 0.005$). The percentage of the oral glucose taken up by the splanchnic tissues also was significantly higher following pioglitazone therapy (46.2 ± 5.1 vs. 33.0 ± 2.8%, $P < 0.005$) (Fig. 3). The increment in SGU was correlated with the decrement in HbA_{1c} (r = 0.57, $P < 0.05$). There was no significant correlation between increased SGU and either the decrease in plasma FFA concentration (r = 0.32, $P > 0.05$) or hepatic fat content (r = 0.02, $P > 0.05$).

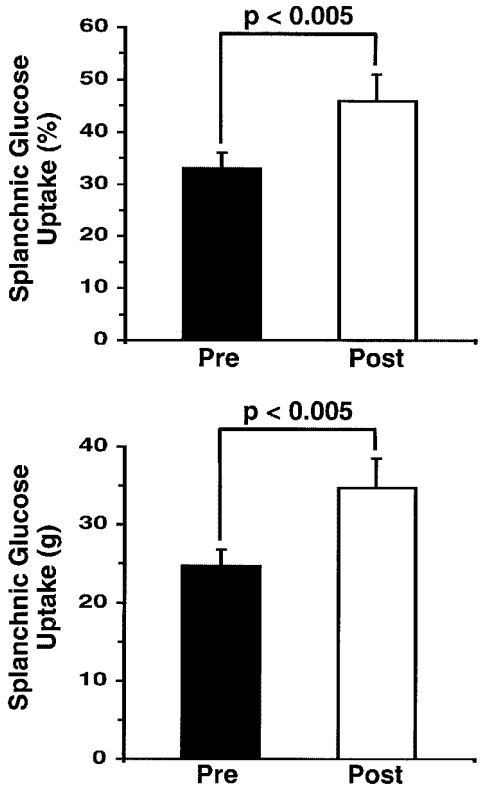

FIG. 3. SGU (absolute and percentage of ingested glucose load) during the oral glucose load–insulin clamp studies performed before (pre) and after (post) pioglitazone treatment.

Hepatic fat content. Pioglitazone therapy resulted in a 47% decrease (Fig. 4) in hepatic fat content (19.6 ± 3.6 to 10.4 ± 2.1%, $P < 0.005$), despite an increase in body weight

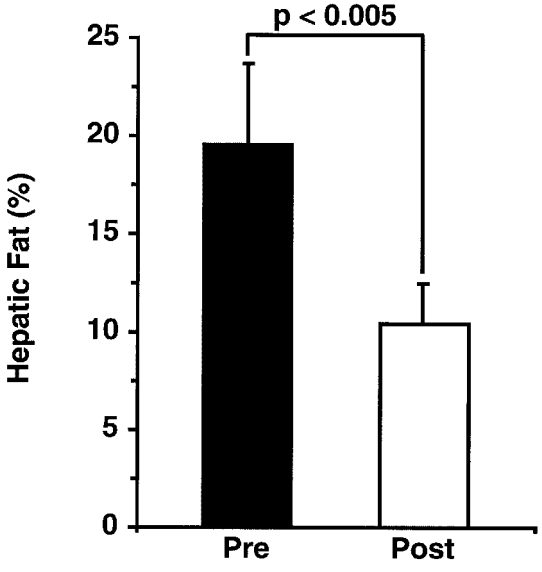

FIG. 4. Hepatic fat content before (pre) and after (post) pioglitazone treatment.

(Δ = 3.0 kg). The decrease in hepatic fat content was associated with a significant improvement in liver function parameters, including both aspartate transaminase and alanine transaminase (Table 1). Before pioglitazone treatment, hepatic fat content was positively correlated (r = 0.65, $P < 0.01$) with EGP during the 150–180 min period of the euglycemic-insulin clamp. EGP was completely suppressed after pioglitazone therapy. Taken collectively, liver fat content before and after pioglitazone treatment still correlated positively with EGP during the insulin clamp (r = 0.63, $P < 0.001$).

DISCUSSION

In the present study, we used the oral glucose load–insulin clamp technique (3,30) and proton spectroscopy to examine the effect of pioglitazone on SGU and hepatic fat content in subjects with type 2 diabetes. The majority of our patients were of Mexican-American descent. Although the metabolic defects in Mexican Americans with type 2 diabetes have been shown to be similar to those in Caucasians (36), one should be careful about extrapolating the conclusions of the present study to other ethnic groups, since 11 of the 14 participants were of Mexican-American ethnicity. The results demonstrate that SGU following glucose ingestion was significantly enhanced after 16 weeks of pioglitazone therapy in patients with type 2 diabetes, while hepatic fat content decreased by 47%. The decrease in hepatic fat content was associated with an improvement in hepatic function, as evidenced by the decline in hepatic transaminases (aspartate transaminase and alanine transaminase). Pioglitazone treatment also was associated with a decline in FPG and FFA concentrations, improved peripheral and hepatic insulin sensitivity, and enhanced insulin-mediated suppression of lipolysis.

The mechanisms by which pioglitazone decreases the hepatic fat content are unclear. The reduction in hepatic fat content observed in the present study occurred despite a significant increase in body weight (3.0 kg). Thiazolidinediones exert their metabolic effects by binding to and activating PPARγ (24,37). PPARγ activation causes preadipocytes to differentiate into mature fat cells and causes the induction of key enzymes involved in lipogenesis (26,38,39). This results in smaller, more insulin-sensitive peripheral adipocytes (38,39) and a shift in fat distribution from visceral to subcutaneous fat depots (26–28). Previous studies from our laboratory (40) have shown that the weight gain following pioglitazone treatment was associated with significant increases in both superficial and deep abdominal subcutaneous adipose depots. Both visceral and hepatic fat content decreased significantly during pioglitazone treatment, although the decrease in visceral fat did not correlate with the decrease in hepatic fat. Consistent with in vitro and in vivo effects in animals (24,38,39), pioglitazone therapy in the present study was associated with a decrease in fasting plasma FFA concentration and improved insulin-mediated suppression of plasma FFA concentrations during the insulin clamp, suggesting enhanced sensitivity of adipocytes to insulin. One could hypothesize that the decrease in circulating plasma FFA (and glucose) concentration(s) resulted in a redistribution of triglyceride from the liver to the peripheral adipocyte. Circulating substrate levels (FFA and glucose) play an important role in hepatic triglyceride synthesis (41). Pioglitazone caused a marked reduction in both fasting plasma FFA and glucose concentrations, and this would be expected to result in a decrease in hepatic triglyceride synthesis. Consistent with this, diabetic patients treated with pioglitazone experienced a significant decline in fasting plasma triglyceride concentration (Table 1). Thiazolidinediones are also peroxisome proliferators, and as such, they increase fat oxidation (42,43). If pioglitazone were to stimulate fat oxidation in the liver, this could contribute to the decrease in hepatic fat content. It should be noted that previous studies from our laboratory did not demonstrate a significant increase in lipid oxidation following pioglitazone therapy in patients with type 2 diabetes (26). However, indirect calorimetry measures whole-body lipid oxidation and cannot examine lipid oxidation in specific tissues, e.g., liver. The decrease in hepatic fat content could be responsible for the improvement in hepatic function, as evidenced by the significant decline in aspartate transaminase and alanine transaminase levels (Table 1). It should be noted that troglitazone, another thiazolidinedione, has been shown to improve liver function in patients with nonalcoholic steatohepatitis (44). Fatty infiltration of the liver and steatonecrosis are well documented in insulin-resistant individuals (45) and in patients with type 2 diabetes (46,47). In the present study, only two subjects had elevated alanine transaminase values (48 and 47 IU/l, respectively). In both these subjects, alanine transaminase decreased into the normal range following pioglitazone treatment. The present results raise the possibility that pioglitazone, as well as other thiazolidinediones, may be useful in the treatment of nonalcoholic hepatic steatonecrosis.

Consistent with previous studies, pioglitazone treatment improved peripheral insulin sensitivity by 33% in type 2 diabetes patients. It should be noted, however, that in the present study, insulin sensitivity was measured at pharmacological levels of hyperinsulinemia. Previous studies from our laboratory have shown that the improvement in insulin sensitivity, when measured at physiological levels of hyperinsulinemia, is very small and cannot explain the major improvement in the oral glucose tolerance test (26,27). One potential explanation for these apparently disparate results is that the thiazolidinediones enhance SGU, which accounts for the disposal of approximately one-third of an ingested glucose load (4,5). In contrast, under physiological conditions of hyperinsulinemia and euglycemia, the splanchnic tissues do not enhance their uptake of glucose above that which is present under basal postabsorptive conditions (2,4,5). The results of the present study support this hypothesis. Thus, following 16 weeks of pioglitazone treatment, splanchnic (primarily hepatic) glucose uptake of an ingested glucose load (75 g) increased significantly, by 10 g (from 24.7 to 34.7 g).

We did not observe a significant correlation between the increment in SGU and the decrement in hepatic fat content. However, the increment in SGU was positively correlated with the decrement in HbA_{1c} following pioglitazone treatment (r = 0.57, $P < 0.05$). In the liver, glucose transport and phosphorylation are mediated via the GLUT2 transporter and glucokinase, respectively. In

animal studies, it has been shown that chronic hyperglycemia decreases glucokinase activity and that restoration of euglycemia results in the normalization of glucokinase activity (48). The results of the present study suggest that amelioration of chronic hyperglycemia by pioglitazone treatment in patients with type 2 diabetes leads to an improvement in SGU.

The oral glucose load–hyperglycemic clamp technique originally was developed in our laboratory to quantitate SGU (3,5). More recently, Ludvik et al. (30) modified the oral glucose load–hyperglycemic clamp technique by administering the oral glucose load during a euglycemic insulin clamp study. This modification has the advantage of providing more reproducible plasma insulin concentrations (Fig. 2), since the arterial plasma glucose concentration is maintained at euglycemic levels. Nonetheless, even though we decreased the exogenous glucose infusion rate to near zero after administration of the oral glucose, we observed a very small rise in plasma glucose concentration during the 180–300 min time period following glucose ingestion before pioglitazone treatment. Following pioglitazone therapy, plasma glucose concentrations during the 180–300 min period of the oral glucose load–insulin clamp were matched for this hyperglycemia in each individual diabetic patient. Importantly, plasma insulin concentrations did not increase from pre–oral glucose load values in response to this small increase in plasma glucose concentration in either the pre- or postpioglitazone studies. The oral glucose load–insulin clamp technique has the additional advantages that it is noninvasive, can be performed repetitively to follow changes in SGU, and circumvents the problems of tracer cycling and non–steady-state conditions that exist with the double-tracer technique. Both the oral glucose load–hyperglycemic clamp and oral glucose load–insulin clamp techniques have been validated by direct comparison with the hepatic vein catheter technique (3,5,30).

The oral glucose load–insulin clamp technique assumes that the absorption of the oral glucose load (75 g) is complete within 4 h and that EGP is completely or nearly completely suppressed. With respect to the first assumption, several studies have demonstrated that an oral glucose load, comparable to that employed in the present study, is completely absorbed within 3–3.5 h (9,30,35). This was confirmed in the present study by return of the exogenous glucose infusion rate during the 380–420 min time period to values that were equal to or greater than the glucose infusion rate at 180 min, i.e., immediately before ingestion of the glucose load. An important assumption of the oral glucose load–insulin clamp technique to measure SGU is that EGP is completely suppressed. Therefore, we chose a high insulin infusion rate (100 mU $\cdot$ m^{-2} $\cdot$ min^{-1}) that produced pharmacological plasma insulin concentrations, resulting in nearly complete suppression of EGP. Since EGP was almost completely suppressed during the last 30 min of the euglycemic insulin clamp, since the plasma insulin concentration remained constant following glucose ingestion, and since portal hyperglycemia following ingestion of the oral glucose load would be expected to further inhibit hepatic glucose production (2,4,8), one can reasonably assume that it remained suppressed during the 4 h following glucose ingestion. A second advantage of the high insulin infusion rate is that it augments peripheral glucose disposal to sufficiently high levels to allow reduction of the exogenous glucose infusion rate, thereby preventing hyperglycemia following glucose ingestion. It should be emphasized that neither physiological nor pharmacological elevations in the plasma insulin concentrations have any stimulatory effect on SGU in humans (2,4,5). The mass-action effect of hyperglycemia to augment SGU is, however, dependent upon maintained portal insulin levels (2).

Because we performed a high-dose (100 mU $\cdot$ m^{-2} $\cdot$ min^{-1}) euglycemic insulin clamp, EGP was suppressed by >90% during the insulin clamp before the start of pioglitazone treatment. Nonetheless, we observed a significant correlation ($r = 0.65$, $P < 0.01$) between hepatic fat content and EGP. Sixteen weeks of pioglitazone therapy caused a significant improvement in insulin-mediated suppression of EGP after pioglitazone treatment. Because of the complete suppression of EGP after pioglitazone treatment, a significant correlation between EGP and hepatic fat content was not detected. However, if the pre- and postpioglitazone results are analyzed collectively, we were able to demonstrate a significant association ($r = 0.63$, $P < 0.001$) between hepatic fat content and EGP. These results are consistent with previous studies showing that impaired suppression of hepatic glucose production by insulin is strongly correlated with increased hepatic fat content in type 2 diabetic patients (21).

In summary, the present results demonstrate that pioglitazone treatment enhances SGU following glucose ingestion, decreases hepatic fat content, and improves liver function tests in patients with type 2 diabetes. The decrease in hepatic fat content following pioglitazone is associated with increased hepatic insulin sensitivity. Improved splanchnic and peripheral glucose uptake and enhanced suppression of EGP after pioglitazone treatment contribute to the improvement in glycemic control in patients with type 2 diabetes.

ACKNOWLEDGMENTS

This work was supported in part by grants from Takeda America, National Institutes of Health Grant DK-24092, and a Veterans Administration Merit Award.

The authors wish to thank the nurses of the General Clinical Research Center for their diligent care of our patients and especially Patricia Wolff, RN, Norma Diaz, BSN, James King, RN, and John Kincade, RN, for carrying out the insulin clamp studies. We gratefully acknowledge the technical assistance of Kathy Camp, Cindy Munoz, and Sheila Taylor. Ms. Lorrie Albarado and Ms. Elva Chapa provided skilled secretarial support in the preparation of this manuscript.

REFERENCES

1. DeFronzo RA: Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Rev* 5:177–269, 1997
2. DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J: Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. *Diabetes* 32:35–45, 1983
3. Ferrannini E, Wahren J, Felig P, DeFronzo RA: Role of fractional glucose extraction in the regulation of splanchnic glucose metabolism in normal and diabetic man. *Metabolism* 29:28–35, 1980
4. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J: Effects of

insulin on peripheral and splanchnic glucose metabolism in non-insulin dependent diabetes mellitus. *J Clin Invest* 76:149–155, 1985
5. DeFronzo RA, Ferrannini E, Hendler R, Wahren J, Felig P: Influence of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose exchange. *Proc Natl Acad Sci U S A* 75:5173–5177, 1978
6. Adkins BA, Myers SR, Hendrick GK, Williams PE, Stevenson RW, Cherrington AD: Importance of the route of intravenous glucose delivery to hepatic glucose balance in the conscious dog. *J Clin Invest* 79:557–565, 1987
7. Myers SR, Biggers DW, Neal DW, Cherrington AD: Intraportal glucose delivery enhances the effects of hepatic glucose load on net hepatic glucose uptake in vivo. *J Clin Invest* 88:158–167, 1991
8. Ferrannini E, Simonson DC, Katz LD, Reichard G, Bevilacqua S, Barrett EJ, Olsson M, DeFronzo RA: The disposal of an oral glucose load in patients with non-insulin dependent diabetes. *Metabolism* 37:79–85, 1988
9. Ludvik B, Nolan JJ, Roberts A, Baloga J, Joyce M, Bell JM, Olefsky JM: Evidence for decreased splanchnic glucose uptake after oral glucose administration in non-insulin-dependent diabetes mellitus. *J Clin Invest* 100:2354–2361, 1997
10. Mitrakou A, Kelley D, Venerman T, Jenssen T, Pangburn T, Reilly J, Gerich J: Contribution of abnormal muscle and liver glucose metabolism to postprandial hyperglycemia in NIDDM. *Diabetes* 39:1381–1390, 1990
11. Basu A, Basu R, Shah P, Vella A, Johnson CM, Nair KS, Jensen MD, Schwenk WF, Rizza RA: Type 2 diabetes impairs splanchnic uptake of glucose but does not alter intestinal glucose absorption during enteral glucose feeding: additional evidence for a defect in hepatic glucokinase activity. *Diabetes* 50:1351–1362, 2001
12. Bajaj M, Berria R, Pratipanawatr T, Kashyap S, Pratipanawatr W, Belfort R, Cusi K, Mandarino L, DeFronzo RA: Free fatty acid-induced peripheral insulin resistance augments splanchnic glucose uptake in healthy humans. *Am J Physiol* 283:E346–E352, 2002
13. Shah P, Vella A, Basu A, Basu R, Adkins A, Schwenk WF, Johnson CM, Nair KS, Jensen MD, Rizza RA: Effects of free fatty acids and glycerol on splanchnic glucose metabolism and insulin extraction in nondiabetic humans. *Diabetes* 51:301–310, 2002
14. Bajaj M, Pratipanawatr T, Berria R, Pratipanawatr W, Kashyap S, Cusi K, Mandarino L, DeFronzo RA: Free Fatty acids reduce splanchnic and peripheral glucose uptake in patients with type 2 diabetes. *Diabetes* 51:3043–3048, 2002
15. Groop LC, Bonadonna RC, Del Prato S, Ratheiser K, Zyck K, DeFronzo RA: Glucose and free fatty acid metabolism in non-insulin-dependant diabetes mellitus: evidence for multiple sites of insulin resistance. *J Clin Invest* 84:205–213, 1989
16. Groop LC, Saloranta C, Shank M, Bonadonna RC, Ferrannini E, DeFronzo RA: The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and non-insulin dependent diabetes mellitus. *J Clin Endocrinol Metab* 72:96–107, 1991
17. Reaven GM: The fourth musketeer: from Alexandre Dumas to Claude Bernard. *Diabetologia* 38:3–13, 1995
18. Banerji MA, Buckley MC, Chaiken RL, Gordon D, Lebovitz HE, Kral JG: Liver fat, serum triglycerides and visceral adipose tissue in insulin-sensitive and insulin-resistant black men with NIDDM. *Int J Obes Relat Metab Disord* 19:846–850, 1995
19. Kawasaki T, Hashimoto N, Kikuchi T, Takahashi H, Uchiyama M: The relationship between fatty liver and hyperinsulinemia in obese Japanese children. *J Pediatr Gastroenterol Nutr* 24:317–321, 1997
20. Goto T, Onuma T, Takebe K, Kral JG: The influence of fatty liver on insulin clearance and insulin resistance in non-diabetic Japanese subjects. *Int J Obes Relat Metab Disord* 19:841–845, 1995
21. Ryysy L, Hakkinen A, Goto T, Vehkavaara S, Westerbacka J, Halavaara J, Yki-Jarvinen H: Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients. *Diabetes* 49:749–758, 2000
22. Wahrenberg H, Lonnqvist F, Arner P: Mechanisms underlying regional differences in lipolysis in human adipose tissue. *J Clin Invest* 84:458–467, 1989
23. Kelley DE, Mandarino LJ: Fuel selection in human skeletal muscle in insulin resistance: a reexamination. *Diabetes* 49:677–683, 2000
24. Spiegelman BM: PPAR-γ: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47:507–514, 1998
25. Miyazaki Y, Glass L, Triplitt C, Matsuda M, Cusi K, Mahankali A, Mahankali S, Mandarino LJ, DeFronzo RA: Effect of rosiglitazone on glucose and non-esterified fatty acid metabolism in type II diabetic patients. *Diabetologia* 44:2210–2219, 2001
26. Miyazaki Y, Mahankali A, Matsuda M, Glass L, Mahankali S, Ferrannini E, Cusi K, Mandarino LJ, DeFronzo RA: Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. *Diabetes Care* 24:710–719, 2001
27. Miyazaki Y, Mahankali A, Matsuda M, Mahankali S, Hardies J, Cusi K, Mandarino LJ, DeFronzo RA: Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. *J Clin Endocrinol Metab* 87:2784–2791, 2002
28. Mori Y, Murakawa Y, Okada K, Horikoshi H, Yokoyama J, Tajima N, Ikeda Y: Effect of troglitazone on body fat distribution in type 2 diabetic patients. *Diabetes Care* 22:908–912, 1999
29. Mayerson AB, Hundal RS, Dufour S, Lebon V, Befroy D, Cline GW, Enocksson S, Inzucchi SE, Shulman GI, Petersen KF: The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. *Diabetes* 51:797–802, 2002
30. Ludvik B, Nolan JJ, Roberts A, Baloga J, Joyce M, Bell JM, Olefsky JM: A noninvasive method to measure splanchnic glucose uptake after oral glucose administration. *J Clin Invest* 95:2232–2238, 1995
31. DeFronzo R, Tobin J, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
32. Bottomley PA: Spatial localization in NMR spectroscopy in vivo. *Ann N Y Acad Sci* 508:333–348, 1987
33. Petersen KF, West AB, Reuben A, Rothman DL, Shulman GI: Noninvasive assessment of hepatic triglyceride content in humans with 13C nuclear magnetic resonance spectroscopy. *Hepatology* 24:114–117, 1996
34. Steele R: Influence of glucose loading and of injected insulin on hepatic glucose output. *Ann N Y Acad Sci* 82:420–430, 1959
35. Kuyumjian J, Kalant N: Absorption of an oral glucose load in the dog. *Horm Metab Res* 18:587–589, 1986
36. Haffner SM, Miettinen H, Stern MP: Insulin secretion and resistance in nondiabetic Mexican Americans and non-Hispanic whites with a parental history of diabetes. *J Clin Endocrinol Metab* 81:1846–1851, 1996
37. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem* 270:12953–12956, 1995
38. Hallakou S, Doare F, Foufelle F, Kergoat M, Guerre-Millo M, Berthault MF, Dugail I, Morin J, Auwerx J, Ferre P: Pioglitazone induces in vivo adipocyte differentiation in obese Zucker *fa/fa* rat. *Diabetes* 46:1393–1399, 1997
39. Lambe KG, Tugwood JD: A human peroxisome-proliferator-activated receptor-gamma is activated by inducers of adipogenesis, including thiazolidinedione drugs. *Eur J Biochem* 239:1–7, 1996
40. Miyazaki Y, Hardies LJ, Wajcberg E, Glass L, Triplitt C, Bajaj M, Cersosimo E, Mandarino LJ, DeFronzo RA: Effect of pioglitazone on liver fat content, abdominal fat distribution and insulin sensitivity in patients with type 2 diabetes mellitus (Abstract). *Diabetes* 51(Suppl. 2):A69, 2002
41. Greenfield M, Kolterman O, Olefsky J, Reaven GM: Mechanism of hypertriglyceridaemia in diabetic patients with fasting hyperglycaemia. *Diabetologia* 18:441–446, 1980
42. Adams M, Montague CT, Prins JB, Holder JC, Smith SA, Sanders L, Digby JE, Sewter CP, Lazer MA, Chatterjee VKK, O'Rahilly S: Activators of peroxisome proliferator-activated receptor gamma have depot-specific effects on human preadipocyte differentiation. *J Clin Invest* 100:3149–3153, 1997
43. Chao L, Marcus-Samuels B, Mason MM, Moitra J, Vinson C, Arioglu E, Gavrilova O, Reitman ML: Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. *J Clin Invest* 106:1221–1228, 2000
44. Caldwell SH, Hespenheide EE, Redick JA, Iezzoni JC, Battle EH, Sheppard BL: A pilot study of thiazolidinedione, troglitazone, in nonalcoholic steatohepatitis. *Am J Gastroenterol* 96:519–525, 2001
45. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN: Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 120:1183–92, 2001
46. Silverman JF, Pories WJ, Caro JF: Liver pathology in diabetes mellitus and morbid obesity: clinical, pathological, and biochemical considerations. *Pathol Annu* 24:275–302, 1989
47. Creutzfeldt W, Frerichs H, Sickinger K: Liver diseases and diabetes mellitus. *Prog Liver Dis* 3:371–407, 1970
48. Nawano M, Oku A, Ueta K, Umebayashi I, Ishirahara T, Arakawa K, Saito A, Anai M, Kikuchi M, Asano T: Hyperglycemia contributes insulin resistance in hepatic and adipose tissue but not skeletal muscle of ZDF rats. *Am J Physiol* 278:E535—E543, 2000