Effects of Combined Exenatide and Pioglitazone Therapy on Hepatic Fat Content in Type 2 Diabetes

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We examined the effects of combined pioglitazone (peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist) and exenatide (GLP-1 receptor agonist) therapy on hepatic fat content and plasma adiponectin levels in patients with type 2 diabetes (T2DM). Twenty-one T2DM patients (age = 52 ± 3 years, BMI = 32.0 ± 1.5, hemoglobin A_{1c} (HbA_{1c}) = 8.2 ± 0.4%) on diet and/or metformin received additional treatment with either pioglitazone 45 mg/day for 12 months (n = 10) or combined therapy with pioglitazone (45 mg/day) and exenatide (10 µg subcutaneously twice daily) for 12 months (n = 11). At baseline, hepatic fat content and plasma adiponectin levels were similar between the two treatment groups. Pioglitazone reduced fasting plasma glucose (FPG) ($P < 0.05$), fasting free fatty acid (FFA) ($P < 0.05$), and HbA_{1c} (Δ = 1.0%, $P < 0.01$), while increasing plasma adiponectin concentration by 86% ($P < 0.05$). Hepatic fat (magnetic resonance spectroscopy (MRS)) was significantly reduced following pioglitazone treatment (11.0 ± 3.1 to 6.5 ± 1.9%, $P < 0.05$). Plasma triglyceride concentration decreased by 14% ($P < 0.05$) and body weight increased significantly (Δ = 3.7 kg). Combined pioglitazone and exenatide therapy was associated with a significantly greater increase in plasma adiponectin (Δ = 193%) and a significantly greater decrease in hepatic fat (12.1 ± 1.7 to 4.7 ± 1.3%) and plasma triglyceride (38%) vs. pioglitazone therapy despite the lack of a significant change in body weight (Δ = 0.2 kg). Hepatic injury biomarkers aspartate aminotransferase and alanine aminotransferase (ALT) were significantly decreased by both treatments; however, the reduction in ALT was significantly greater following combined pioglitazone and exenatide therapy. We conclude that combined in patients with T2DM, pioglitazone and exenatide therapy is associated with a greater reduction in hepatic fat content as compared to the addition of pioglitazone therapy (Δ = 61% vs. 41%, $P < 0.05$).

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INTRODUCTION

Nonalcoholic fatty liver disease is common in type 2 diabetic patients. Type 2 diabetic patients have 80% more liver fat than age-, weight-, and sex-matched nondiabetic subjects (1). Insulin resistance in type 2 diabetics is associated with chronic hyperinsulinemia, hyperglycemia, and elevated plasma free fatty acids (FFAs) resulting in enhanced hepatic lipogenesis. Individuals with a nonalcoholic fatty liver disease are more likely to have excess intra-abdominal (2) fat as well as a reduction in circulating plasma adiponectin levels (3). In rodents, hypoadiponectinemia is associated with a reduction in hepatic AMP-activated protein kinase activity and subsequent defects in mitochondrial fat oxidation as well as increased hepatic triglyceride content and associated hepatic insulin resistance (4).

We (3,5), and others (6), have previously shown that type 2 diabetes (T2DM) is characterized by increased hepatic fat content, hypoadiponectinemia, and hepatic insulin resistance. Treatment with peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists (thiazolidinediones) in patients with T2DM is associated with a reduction in plasma FFA levels and FFA turnover, a shift in fat distribution from visceral and hepatic to subcutaneous depots, improved hepatic and peripheral (muscle) insulin sensitivity, and a two to threefold increase in plasma adiponectin despite an increase in body weight (3,5,7). Furthermore, the increase in adiponectin following PPAR-γ agonist treatment correlates with the decrease in hepatic fat content as well as hepatic insulin resistance in type 2 diabetic patients. Of note, positive relationships

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between increased hepatic fat content and various measures of insulin resistance have been observed in humans, independent of BMI (3,5). These novel observations were followed by recent studies examining the role of PPAR-γ agonists in the pharmacotherapy of nonalcoholic steatohepatitis (NASH) (8–10).

Weight loss in humans with nonalcoholic fatty liver disease is associated with a decrease in hepatic fat content (11). Exenatide, a GLP-1 receptor agonist that enhances glucose-dependent insulin secretion and glucose-dependent suppression of inappropriately high glucagon secretion, improves glycemic control in patients with T2DM (12). Exenatide-induced weight loss in preclinical experiments subsequently fostered interest in the ability of such agents to diminish appetite and reduce weight gain in overweight human subjects. Healthy nondiabetic subjects, obese subjects as well as patients with T2DM show a significant reduction in appetite and meal ingestion following GLP-1 treatment (13,14). In patients with T2DM, adjunctive exenatide treatment for 3 years resulted in a progressive reduction in body weight, improvement in glycemic control and improvements in the hepatic injury biomarkers, aspartate aminotransferase, and alanine aminotransferase (ALT) (15). Furthermore, thiazolidinedione-induced weight gain can be ameliorated by the addition of exenatide therapy in patients with T2DM (12).

However, the effect of combined exenatide and thiazolidinedione therapy on hepatic fat content has not been previously studied. This is important given the recent observation that exenatide increases plasma adiponectin levels and expression and reduces hepatic fat content in rodents (16–18). Furthermore, we have shown that the increase in adiponectin levels following exendin-4 therapy in a rodent model of obesity may be independent of changes in weight (16). The current study was designed to examine the effect of combined exenatide and pioglitazone therapy on hepatic fat content in patients with T2DM on diet and/or metformin therapy.

METHODS AND PROCEDURES

Subjects

Twenty four patients with T2DM on diet and/or metformin therapy were enrolled in the study of which 21 patients (age = 52 ± 3 years, BMI = 32.0 ± 1.5, hemoglobin A_{1c} (HbA_{1c}) = 8.2 ± 0.4%) completed the entire study. Metformin-treated diabetic patients were included because metformin therapy does not alter hepatic fat content or plasma adiponectin levels in patients with T2DM (19). The dosage of metformin was not altered throughout the duration of the study. Patients who had received insulin, sulfonylureas, sitagliptin, exenatide or a thiazolidinedione, within the previous 3 months were excluded. Entry criteria included: age = 30–70 years, stable body weight (±2 lbs) for at least 3 months before study, fasting plasma glucose (FPG) = 126–260 mg/dl. 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. Patients with ALT or aspartate aminotransferase greater than 2.5 times upper limit of normal were excluded from the study. 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 Baylor College of Medicine approved the protocol.

Study design

Three weeks before study, 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 prior to start of the study medication(s), all subjects received: (i) baseline measurements of FPG, plasma adiponectin, FFA, fasting plasma insulin, fasting plasma lipids, liver function tests, and HbA_{1c}, and (ii) measurement of liver fat content with magnetic resonance spectroscopy (MRS) (3,5). All studies were started at 0800 h following a 10–12-h overnight fast. Following completion of these studies, subjects were randomized (using a table of random numbers) to participate in one of the two treatment arms (i) pioglitazone 30 mg/day orally for 2 weeks followed by pioglitazone 45 mg/day orally for 50 weeks (n = 10) or (ii) exenatide 5 μg injected subcutaneously twice daily and pioglitazone 30 mg/day orally for 2 weeks followed by exenatide 10 μg subcutaneously twice daily and pioglitazone 45 mg/day orally for 50 weeks (n = 11) in an open label study. Seventeen subjects were taking a stable dose of metformin for at least 3 months before study, and four subjects were treated with diet alone. Two patients in each treatment group were not on metformin therapy at the time of randomization and did not receive metformin therapy during the entire duration of the study. All subjects underwent repeat measurement of fasting plasma insulin, FFA, adiponectin, and determination of hepatic fat content by MRS at the end of 12 months. During the entire 12-month treatment period, subjects returned to the Clinical Research Center every 4 weeks at 0800 h following an overnight fast for measurement of FPG, body weight, and blood pressure. On each visit, dietary adherence was reinforced and medication compliance was assessed. Fasting plasma lipids (total cholesterol, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol), liver function tests, and HbA_{1c} were measured every 3 months.

Liver fat content (MRS)

Liver fat content was assessed by MRS as previously described (3,5). Briefly, localized ^1H nuclear magnetic resonance spectra of the liver were obtained on a 1.5 T magnetic resonance imaging scanner using a standard 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/echo time = 130 ms/15 ms·160 degrees; slice thickness = 7 mm; field of view = 44 cm × 45 cm; number of excitations = 1; and an 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 will be accomplished by using a point resolved spectroscopy sequence. The imaging parameters for point resolved spectroscopy sequence were as follows: repetition time/echo time = 1500 ms/54 ms·90 degree; 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 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 landmarks visualizing images.

After line broadening, phase and baseline correction, the peak area of the water at 4.77 ppm, and fat resonance 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 was processed using the operating system software.

Hepatic fat percentage was calculated by dividing (100 × fat resonance) by the sum of fat resonance and peak area of the water. This technique is highly reproducible, with a coefficient of variation less than 2% when the same subjects were studied on eight 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 (20).

Table 1 Anthropometric and metabolic parameters in 21 T2DM patients at baseline, after 12 months of pioglitazone (*N* = 10) therapy (PIO-POST), and after 12 months of exenatide plus pioglitazone (*N* = 11) combination therapy (PIO + EX-POST)

	PIO-PRE	PIO-POST	PIO + EX-PRE	PIO + EX-POST
Body weight (kg)	93.1 ± 7.5	96.8 ± 7.3*	95.5 ± 5.0	95.7 ± 5.1***
BMI	29.7 ± 1.9	30.9 ± 2.1*	34.1 ± 1.3	34.3 ± 1.5
HbA_{1c} (%)	8.3 ± 0.4	7.3 ± 0.3**	8.1 ± 0.5	6.8 ± 0.4**
Fasting plasma glucose (mg/dl)	197 ± 21	155 ± 17*	167 ± 18	119 ± 11**
Fasting plasma insulin (μU/ml)	13 ± 1	11 ± 1*	15 ± 1	13 ± 1*
Fasting plasma FFA (μmol/l)	487 ± 46	331 ± 30*	603 ± 58	369 ± 42**
Total cholesterol (mg/dl)	176 ± 9	181 ± 11	189 ± 14	175 ± 11
LDL cholesterol (mg/dl)	96 ± 7	101 ± 10	118 ± 10	111 ± 9
HDL cholesterol (mg/dl)	44 ± 2	48 ± 3	48 ± 3	54 ± 4*
Triglycerides (mg/dl)	192 ± 25	165 ± 19*	136 ± 13	85 ± 7**,†
ALT (U/l)	25 ± 2	19 ± 2*	35 ± 6	18 ± 2**,***
AST (U/l)	20 ± 2	16 ± 2*	25 ± 5	16 ± 1*

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FFA, free fatty acid; HDL, high-density lipoprotein; HbA_{1c}, hemoglobin A_{1c}; LDL, low-density lipoprotein; T2DM, type 2 diabetes.
*$P < 0.05$ vs. PRE. **$P < 0.01$ vs. PRE. ***$P < 0.05$ vs. PIO, change from baseline. †$P < 0.01$ vs. PIO, change from baseline.

Analytical determinations

Plasma insulin was measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Plasma FFA concentration was determined by an enzymatic calorimetric quantification method (Wako Chemicals, Nuess, Germany). Plasma adiponectin concentration was measured by ELISA (LINCO Research, St Charles, MO).

Statistical analysis

Between groups (i.e., pioglitazone/exenatide combination therapy vs. pioglitazone therapy) comparisons were made using independent Student's *t*-test. Pre- and post-treatment within a group were analyzed using paired *t*-test. Pearson correlation coefficients were calculated to determine the relationship between selected variables. Data are presented as mean ± s.e.m. A *P* value <0.05 was considered to be statistically significant. All statistical analysis were performed using SAS (Cary, NC).

RESULTS

Metabolic parameters are shown in Table 1. After 12 months of therapy, pioglitazone reduced FPG ($P < 0.05$), FFA ($P < 0.05$), and insulin concentrations ($P < 0.05$) and improved glycemic control HbA_{1c} ($P < 0.01$). Total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol did not change, while fasting plasma triglyceride declined ($P < 0.05$) declined after 12 months of pioglitazone therapy. Body weight (Δ = 3.7 kg) increased ($P < 0.05$) after 12 months of pioglitazone therapy.

Following the addition of pioglitazone and exenatide therapy for 12 months, FPG ($P < 0.01$), FFA ($P < 0.01$), and insulin concentrations ($P < 0.05$), HbA_{1c} ($P < 0.01$), and fasting plasma triglycerides ($P < 0.01$) decreased significantly, while high-density lipoprotein cholesterol increased ($P < 0.05$). Body weight (Δ = 0.2 kg) did not change significantly following 12 months of pioglitazone and exenatide therapy. In comparison to pioglitazone therapy, pioglitazone and exenatide therapy was associated with a greater decrease in triglycerides ($P < 0.01$, change from baseline) in the absence of significant alterations in body weight.

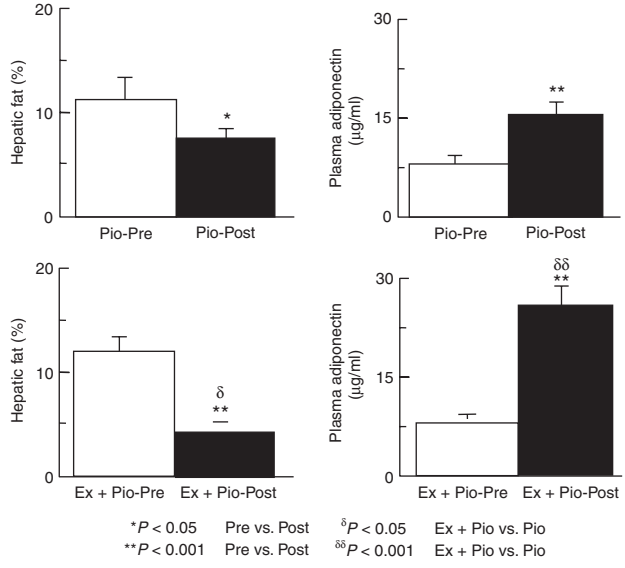

Figure 1 Effect of pioglitazone (Pio) therapy and combined pioglitazone and exenatide (Ex + Pio) therapy on hepatic fat content and plasma adiponectin levels in patients with type 2 diabetes.

Plasma adiponectin concentration

Plasma adiponectin increased significantly after 12 months of pioglitazone therapy (8.5 ± 0.8 to 15.8 ± 1.4 μg/ml, $P < 0.001$) (Figure 1). After the addition of pioglitazone and exenatide for 12 months, plasma adiponectin levels increased almost threefold (7.9 ± 0.9 to 23.2 ± 2.7 μg/ml, $P < 0.001$). In comparison to pioglitazone therapy, pioglitazone and exenatide therapy was associated with a greater increase in

plasma adiponectin concentration (86% vs. 193%, $P < 0.001$) (Figure 1).

Hepatic fat content

At baseline, hepatic fat content was similar between treatment groups. Pioglitazone therapy (Figure 1) reduced hepatic fat content (11.0 ± 3.1 to 6.5 ± 1.9%, $P < 0.05$) after 12 months of treatment. Following the addition of exenatide and pioglitazone therapy, hepatic fat content declined significantly (12.1 ± 1.7 to 4.7 ± 1.3%, $P < 0.001$) after 12 months of treatment. The reduction in hepatic fat content was significantly greater (Figure 1) following the addition of pioglitazone and exenatide therapy in comparison to pioglitazone therapy. Liver transaminases were significantly reduced after 12 months of pioglitazone therapy as well as combined pioglitazone and exenatide therapy (Table 1). In comparison to pioglitazone therapy, pioglitazone and exenatide therapy was associated with a greater decrease in ALT ($P < 0.05$).

Taken collectively, before and after therapy, liver fat content (in patients on pioglitazone and exenatide therapy) correlated with plasma adiponectin concentration ($r = -0.54$, $P < 0.01$), plasma triglyceride levels ($r = 0.75$, $P < 0.001$), ALT ($r = 0.68$, $P < 0.001$), aspartate aminotransferase ($r = 0.55$, $P < 0.01$), and fasting plasma FFA ($r = 0.51$, $P < 0.01$), but did not correlate with FPG ($r = 0.38$, $P > 0.05$).

DISCUSSION

The results of the present study demonstrate that the addition of combined pioglitazone and exenatide therapy was associated with a significantly greater reduction in hepatic fat (Δ = 61% vs. 41%, $P < 0.05$) as well as a greater increase in circulating adiponectin in patients with T2DM as compared to the addition of pioglitazone alone. Furthermore, addition of pioglitazone and exenatide therapy was associated with the lack of a significant alteration in body weight as compared to the weight gain associated with pioglitazone therapy.

It could be argued that exenatide, when combined with pioglitazone, acted more as an appetite suppressant and thereby accounted for these improved biochemical parameters. At baseline, hepatic fat content and plasma adiponectin levels were similar between the two treatment groups (Figure 1). Our patients were on a weight-maintaining diet throughout the duration of the study and dietary adherence was reinforced at each visit. However, patient food intake can rarely be controlled, and reduced caloric intake following combined exenatide and pioglitazone therapy is certainly possible. Indeed, PPAR-γ agonists reduce leptin expression and increase food intake in rodents (21) and the ability of exenatide to reduce appetite and ameliorate thiazolidinedione-induced weight gain may explain the greater reduction in hepatic fat content observed with combined exenatide and pioglitazone therapy. Combined pioglitazone and exenatide therapy was also associated with a greater increase in plasma adiponectin levels. Adiponectin activates AMP-activated protein kinase and enhanced mitochondrial fat oxidation and is thought to be an important mediator of the metabolic effects of thiazolidinediones in the liver (4,22,23). When adiponectin is replaced in rodent models of NASH, there is a significant reduction in steatosis (24). In contrast to thiazolidinedione therapy, modest weight loss following reduced caloric intake is not associated with significant alterations in plasma adiponectin levels in humans (25). Furthermore, strong relationships between decreased hepatic fat content and enhanced plasma adiponectin levels have been observed in humans following thiazolidinedione treatment, independent of BMI (3). Similarly, in a rodent model of obesity, we have shown that exendin-4 may directly contribute to increasing adiponectin levels *in vivo* independent of weight and body composition (16). Recent work suggests that exendin-4 directly induces adiponectin expression through protein kinase A pathway in cultured 3T3-L1 adipocytes (26). Consistent with these observations, plasma adiponectin levels before and after (taken collectively) combined pioglitazone and exenatide treatment correlated negatively with hepatic fat content ($r = -0.54$, $P < 0.01$). The molecular mechanism(s) responsible for exenatide-induced augmentation of circulating adiponectin levels in patients on combined therapy needs to be examined in future studies.

Finally, a direct effect of exenatide on hepatic lipogenesis/lipid oxidation can not be ruled out (16,18). Ding *et al.* reported (18) that exendin-4 appears to effectively reverse hepatic steatosis in ob/ob mice. Furthermore, GLP-1–treatment resulted in a significant reduction in mRNA expression of stearoyl-CoA desaturase 1 and genes associated with fatty acid synthesis; the converse was true for genes associated with fatty acid oxidation in hepatocytes. In DIO mice, we similarly observed that exendin-4 reduced gene expression of key lipogenic enzymes in the liver in association with a reduction in hepatic steatosis (16). Exenatide could be a cause of these observations, due to its direct effects or an indirect effect secondary to improved insulin sensitivity and/or decreased steatosis in association with reduced weight. The presence of the known GLP-1 receptor on hepatocytes is controversial, but there is evidence of specific binding of GLP-1 and activation of signal transduction cascades in hepatocytes (16,18,27,28) and further work is needed to determine the mechanism(s) by which exenatide can affect hepatocyte gene expression.

Exenatide therapy in patients with T2DM is associated with a favorable effect on triglyceride and high-density lipoprotein cholesterol levels and a reduction in body weight in T2DM. The greatest improvement in lipids were observed in patients who had the greatest weight reduction suggesting potential benefits of exenatide beyond improved glycemic control (15). Consistent with these observations, we observed a significantly greater reduction in plasma triglyceride levels in patients who received exenatide and pioglitazone in comparison to those who received pioglitazone (Δ = 38% vs. 14%, $P < 0.01$). The greater reduction in triglycerides is noteworthy given that baseline triglyceride concentrations were significantly lower in those patients who received combined therapy (136 ± 13 vs. 192 ± 25 mg/dl).

Previous studies have shown a 40–50% reduction in hepatic fat content following thiazolidinedione therapy in patients with T2DM (5,7). Consistent with the results of those studies, we demonstrated a 41% reduction in hepatic fat content following pioglitazone treatment. Furthermore, studies have also shown a similar improvement in hepatic steatosis in patients with NASH (9,10) as well as histologic improvements in hepatic necroinflammation following thiazolidinedione therapy (8–10). However, recent placebo-controlled clinical trials have not shown a significant benefit of pioglitazone treatment on histologic hepatic fibrosis score in NASH patients (10,29). Given the probable lack of an effect of pioglitazone therapy on hepatic fibrosis, future studies examining the effect of combined pioglitazone and exenatide therapy on improvement in histologic features of NASH are necessary.

Three patients who received pioglitazone and exenatide combination therapy complained of significant nausea which was self-limiting and did not require discontinuation from the study; none of the patients complained of severe nausea or vomiting. Besides weight gain observed in the pioglitazone-treated patients, lower extremity edema was seen in two of the ten patients. Congestive heart failure was not observed in any of the study patients although we excluded patients with known congestive heart failure (NYHA class I–IV) from participation in the study.

In conclusion, the addition of combined pioglitazone and exenatide therapy was associated with a significantly greater reduction in hepatic fat content as well as a significantly greater increase in plasma adiponectin concentration in patients with T2DM as compared to the addition of pioglitazone alone. The greater reduction in hepatic fat content was associated with the lack of a significant alteration in body weight as compared to the weight gain associated with the addition of pioglitazone therapy. These results suggest that combined thiazolidinedione and exenatide therapy is likely to be of additional therapeutic benefit in treating nonalcoholic fatty liver disease in patients with T2DM as compared to thiazolidinediones therapy. However, we would strongly caution that the results of this study are preliminary and the effects of combined exenatide and thiazolidinedione therapy in a large group of patients with NASH remains to be studied.

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REFERENCES

1. Kotronen A, Juurinen L, Hakkarainen A *et al*. Liver fat is increased in type 2 diabetic patients and underestimated by serum alanine aminotransferase compared with equally obese nondiabetic subjects. *Diabetes Care* 2008;31:165–169.
2. Kotronen A, Westerbacka J, Bergholm R, Pietiläinen KH, Yki-Järvinen H. Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* 2007;92:3490–3497.
3. Bajaj M, Suraamornkul S, Piper P *et al*. Decreased plasma adiponectin concentrations are closely related to hepatic fat content and hepatic insulin resistance in pioglitazone-treated type 2 diabetic patients. *J Clin Endocrinol Metab* 2004;89:200–206.
4. Yamauchi T, Kamon J, Waki H *et al*. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001;7:941–946.
5. Bajaj M, Suraamornkul S, Pratipanawatr T *et al*. Pioglitazone reduces hepatic fat content and augments splanchnic glucose uptake in patients with type 2 diabetes. *Diabetes* 2003;52:1364–1370.
6. Ryysy L, Häkkinen AM, Goto T *et al*. 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* 2000;49:749–758.
7. Mayerson AB, Hundal RS, Dufour S *et al*. The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. *Diabetes* 2002;51:797–802.
8. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. *Hepatology* 2003;38:1008–1017.
9. Promrat K, Lutchman G, Uwaifo GI *et al*. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology* 2004;39:188–196.
10. Belfort R, Harrison SA, Brown K *et al*. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006;355:2297–2307.
11. Petersen KF, Dufour S, Befroy D *et al*. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 2005;54:603–608.
12. Zinman B, Hoogwerf BJ, Durán García S *et al*. The effect of adding exenatide to a thiazolidinedione in suboptimally controlled type 2 diabetes: a randomized trial. *Ann Intern Med* 2007;146:477–485.
13. Gutzwiller JP, Drewe J, Göke B *et al*. Glucagon-like peptide-1 promotes satiety and reduces food intake in patients with diabetes mellitus type 2. *Am J Physiol* 1999;276:R1541–R1544.
14. Toft-Nielsen MB, Madsbad S, Holst JJ. Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. *Diabetes Care* 1999;22:1137–1143.
15. Klonoff DC, Buse JB, Nielsen LL *et al*. Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years. *Curr Med Res Opin* 2008;24:275–286.
16. Samson SL, Gonzalez EV, Yechoor V *et al*. Gene therapy for diabetes: metabolic effects of helper-dependent adenoviral exendin 4 expression in a diet-induced obesity mouse model. *Mol Ther* 2008;16:1805–1812.
17. Li L, Yang G, Li Q *et al*. Exenatide prevents fat-induced insulin resistance and raises adiponectin expression and plasma levels. *Diabetes Obes Metab* 2008;10:921–930.
18. Ding X, Saxena NK, Lin S *et al*. Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. *Hepatology* 2006;43:173–181.
19. Tiikkainen M, Häkkinen AM, Korsheninnikova E *et al*. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. *Diabetes* 2004;53:2169–2176.
20. 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* 1996;24:114–117.
21. De Vos P, Lefebvre AM, Miller SG *et al*. Thiazolidinediones repress ob gene expression in rodents via activation of peroxisome proliferator-activated receptor gamma. *J Clin Invest* 1996;98:1004–1009.
22. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001;7:947–953.
23. Yamauchi T, Kamon J, Minokoshi Y *et al*. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002;8:1288–1295.

24. Xu A, Wang Y, Keshaw H *et al*. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003;112:91–100.
25. Abbasi F, Chang SA, Chu JW *et al*. Improvements in insulin resistance with weight loss, in contrast to rosiglitazone, are not associated with changes in plasma adiponectin or adiponectin multimeric complexes. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R139–R144.
26. Kim Chung le T, Hosaka T, Yoshida M *et al*. Exendin-4, a GLP-1 receptor agonist, directly induces adiponectin expression through protein kinase A pathway and prevents inflammatory adipokine expression. *Biochem Biophys Res Commun* 2009;390:613–618.
27. Redondo A, Trigo MV, Acitores A, Valverde I, Villanueva-Peñacarrillo ML. Cell signalling of the GLP-1 action in rat liver. *Mol Cell Endocrinol* 2003;204:43–50.
28. Gupta NA, Mells J, Dunham RM *et al*. Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis *in vitro* by modulating elements of the insulin signaling pathway. *Hepatology* 2010;51:1584–1592.
29. Sanyal AJ, Chalasani N, Kowdley KV *et al*.; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010;362:1675–1685.