Ameliorated Hepatic Insulin Resistance Is Associated with Normalization of Microsomal Triglyceride Transfer Protein Expression and Reduction in Very Low Density Lipoprotein Assembly and Secretion in the Fructose-fed Hamster*

Received for publication, May 9, 2002
Published, JBC Papers in Press, June 4, 2002, DOI 10.1074/jbc.M204568200

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To determine whether reduction of insulin resistance could ameliorate fructose-induced very low density lipoprotein (VLDL) oversecretion and to explore the mechanism of this effect, fructose-fed hamsters received placebo or rosiglitazone for 3 weeks. Rosiglitazone treatment led to normalization of the blunted insulin-mediated suppression of the glucose production rate and to a ~2-fold increase in whole body insulin-mediated glucose disappearance rate (p < 0.001). Rosiglitazone ameliorated the defect in hepatocyte insulin-stimulated tyrosine phosphorylation of the insulin receptor, IRS-1, and IRS-2 and the reduced protein mass of IRS-1 and IRS-2 induced by fructose feeding. Protein-tyrosine phosphatase 1B levels were increased with fructose feeding and were markedly reduced by rosiglitazone. Rosiglitazone treatment led to a ~50% reduction of VLDL secretion rates (p < 0.05) in vivo and ex vivo. VLDL clearance assessed directly in vivo was not significantly different in the FR (fructose-fed + rosiglitazone-treated) versus F (fructose-fed + placebo-treated) hamsters, although there was a trend toward a lower clearance with rosiglitazone. Enhanced stability of nascent apolipoprotein B (apoB) in fructose-fed hepatocytes was evident, and rosiglitazone treatment resulted in a significant reduction in apoB stability. The increase in intracellular mass of microsomal triglyceride transfer protein seen with fructose feeding was reduced by treatment with rosiglitazone. In conclusion, improvement of hepatic insulin signaling with rosiglitazone, a peroxisome proliferator-activated receptor γ agonist, is associated with reduced hepatic VLDL assembly and secretion due to reduced intracellular apoB stability.

The typical dyslipidemia of insulin-resistant states and Type 2 diabetes consists of hypertriglyceridemia due to VLDL overproduction, low high density lipoprotein cholesterol, and small dense low density lipoprotein particles (1). Elevated plasma free fatty acid (FFA) flux from peripheral and intra-abdominal adipose tissue depots due to resistance to the insulin antilipolytic and esterification effect in adipose tissue is felt to play an important role in driving VLDL assembly and secretion in insulin resistant states (2–4). Nevertheless, previous studies in humans suggest that insulin also has an important direct effect on the liver in controlling VLDL secretion (5–7).

Rat and mouse models of insulin resistance and type 2 diabetes have provided important insights into the molecular mechanisms of insulin resistance. These animal models may not, however, be ideal for the study of human lipoprotein disorders because, unlike humans, their livers secrete both apoB48 and apoB100-containing VLDL, and they do not necessarily develop VLDL oversecretion as the basis for their hypertriglyceridemia (8, 9). Unlike livers from rat or mouse, the liver of the golden Syrian hamster secretes only apoB100-containing VLDL, and its lipoprotein metabolism more closely resembles that of humans (10). We have shown that insulin resistance in the fructose-fed golden Syrian hamster is associated with mild hypertriglyceridemia, VLDL-apoB oversecretion, increased intracellular apoB-containing lipoprotein particle stability, and increased expression of microsomal triglyceride transfer protein (MTP) (11). The present studies were conducted to explore the effect of improving insulin sensitivity in this insulin-resistant animal model by treatment with rosiglitazone, a peroxisome proliferator-activated receptor γ agonist and insulin sensitizer and to gain further insight into the molecular mechanisms of VLDL oversecretion in insulin resistant states.

EXPERIMENTAL PROCEDURES

Animals and Study Protocols

Male Syrian golden hamsters (Charles River, Quebec, Canada) were housed in pairs and given free access to food and water. After 7 days...
acclimatization, animals were placed on a fructose-enriched diet (ham-
ster diet with 60% fructose, pelleted, Dyets Inc., Bethlehem, PA) for 5
weeks. After 2 weeks of feeding with the fructose-enriched diet, the
animals were randomized to receive either rosiglitazone (20 μmol/kg/
day) (GlaxoSmithKline) or water only given once daily by gavage for
the remaining 3 weeks of the fructose feeding period. At the end of the
5 weeks, the fructose-fed (F) and fructose-fed + rosiglitazone-treated (FR)
animals underwent either one of the three in vivo protocols described below or isolation of hepatocytes for the ex vivo protocols.

In addition, some animals remained on regular chow for 5
weeks to serve as normal controls.

### In Vivo Protocols

#### Euglycemic Hyperinsulinemic Clamp Studies—Studies were performed as previously described (11) with the following modifications.

Catheters were kept patent overnight with 4% heparin in normal saline (Hepalet, Organon Teknika, 1000 IU/ml). At 8:00 a.m. the morning after insertion of femoral vein and arterial catheters, a primed (10 μCi) constant (0.1 μCi/min) infusion of high performance liquid chromatography-purified [3-3H]glucose (PerkinElmer Life Sciences) was started (time, 90 min) (12). [3-3H]Glucose was added to the 20% dextrose
Infusate to minimize the decline in glucose specific activity during the clamp.

After 75 min of equilibration at time 0 min, a primed (80
milliunits/kg) constant insulin infusion (8 milliunits/kg/min in 0.1% bovine serum albumin in normal saline) (Humulin R, Eli Lilly, Canada) was started, and a 2D0% infusion was adjusted at 10-min intervals to maintain blood glucose at base-line level. Blood samples (0.25 ml) were taken from the arterial line at times 15, 0, 90, 100, 110, and 120 min of the clamp for measurement of blood glucose, [3-3H]glucose specific activity (SA), and plasma insulin levels. There was no significant
decay in hematocrit throughout the study. Endogenous glucose produc-
tion (Ra) was calculated as the endogenous rate of appearance measured with [3-3H]glucose using a modified one-compartment model (13).

Insulin-mediated glucose disappearance (ΔRd) was the rate of disapp-
pearance measured with [3-3H]glucose during the clamp minus the mean base-line Rd level. Data were smoothed with the optimal seg-
ments routine (14) using the optimal error algorithm (15). Because euglycemia was maintained in one hamster of the FR group, this animal was not included in the analysis of these experiments.

#### VLDL Secretion Studies—One day before these studies, cathers
were inserted into the femoral vein and artery of F (n = 10) and FR animals (n = 9) of similar weight (134 ± 3 g versus 132 ± 2 g, respectively, p = 0.64) and chow-fed controls (n = 5) as previously described (11). VLDL-apoB and VLDL-triglyceride (TG) secretion rates were measured in the fasting state (12 h) after intravenous injection of Triton WR-1339 (Sigma) as previously described (11). The total blood volume of the samples drawn was less than 1.5 ml per animal during the experiment, and there was no significant decline in hematocrit.

#### VLDL Clearance Studies—Because the Triton method does not allow direct assessment of VLDL clearance, the following studies were performed after a 12-h fast in 7 F and 8 FR animals of similar weight (129 ± 6 g versus 126 ± 4 g, respectively, p = 0.68). Catheters were inserted the day before these studies into the femoral vein and artery. A bolus (20 μCi) of [2-3H]glycerol (PerkinElmer Life Sciences) was injected intravenously, and blood samples were collected at times 10, 15, 20, 30, 35, 40, and 50 min after the injection to measure VLDL-TG levels and to determine the rate of decline of VLDL-TG [2-3H]glycerol SA. The fractional clearance rate of VLDL-TG [2-3H]glycerol SA over time, determined by linear regression over the linear portion of the down-slope, as previously described (16).

### Ex Vivo Protocols

#### Liver Perfusion and Isolation of Primary Hamster Hepatocytes—
After an overnight fast, the liver of animals from the F and FR groups was perfused under anesthesia, and hepatocytes released from digested liver tissue were transferred into culture medium and seeded in collagen-coated plates as previously described (11).

#### OTHER LABORATORY METHODS

Measurement of glucose, insulin, FFA, TG, apoB, [3-3H]glucose SA, and VLDL isolation were performed as previously described (7, 11). VLDL-TG [2-3H]glycerol SA (dpm/mg) was determined as previously described (5).

#### Statistical Analysis

All the values are reported as mean ± S.E. unless otherwise stated. For the euglycemic clamp studies, two-way analysis of variance was used to compare the glucose, insulin, Ra, and ΔRd curves of the F, FR, and control chow-fed groups at base line and during the last 30 min of the clamp, and the difference between the three groups was assessed by post-hoc analysis using Scheffe test. A two-tailed unpaired homoscedastic t test was used to compare all the other quantitative parameters between F and FR hamsters and between F and control chow-fed hamsters. A p value less than 0.05 was considered to be significant.

### RESULTS

#### Effect of Rosiglitazone Treatment on Body Weight, Plasma Insulin, FFA, TG, and Glucose (Table I)

Because of constraints imposed by the small blood volume of the animals, not all variables were measured on each animal undergoing the various experiments. Fasting plasma insulin was significantly lower (p = 0.02) in the FR and control chow-fed group than in the F group. Total plasma TG levels tended to be lower (by ~30%, p = 0.16) after rosiglitazone treatment.
versus the fructose-fed hamsters and were identical to TG levels in the control chow-fed hamsters. All other variables were not significantly different.

**Treatment of Fructose-fed Hamsters with Rosiglitazone Ameliorates Whole-body Insulin Sensitivity and Improves Hepatocyte Insulin Signaling**

**Euglycemic Hyperinsulinemic Clamp Studies**—Plasma glucose (Fig. 1A) was higher in the F versus FR animals at base line (4.4 ± 0.3 versus 3.2 ± 0.2 mmol/liter, p < 0.03) and during the last 30 min of the clamp (4.0 ± 0.3 mmol/liter versus 3.0 ± 0.1 mmol/liter, p < 0.001) but was kept constant by design throughout the clamp. Hamsters fed a normal chow diet had intermediate glucose levels at base line (3.7 ± 0.2 mmol/liter) and during the last 30 min of the clamp (3.4 ± 0.1 mmol/liter, p < 0.001 versus the F group). The insulin levels (Fig. 1B) were similar throughout the clamp in the F, FR, and the control chow-fed group. Glucose SA (not shown) remained constant in the last 30 min of the clamp in the three groups. The endogenous glucose production rate (Ra) (Fig. 1C) was significantly higher in the F versus FR animals at base line (80.6 ± 12.2 versus 54.0 ± 11.1 μmol/kg/min, p < 0.001) and throughout the clamp (51.9 ± 14.3 versus 10.7 ± 7.0 μmol/kg/min, p < 0.001). Treatment of the F animals with rosiglitazone resulted in normalization of Ra at base line and during the clamp (p = NS versus control chow-fed group) and also led to normalization of

![Euglycemic hyperinsulinemic clamp. Shown are blood glucose levels (A), plasma insulin levels (B), endogenous glucose appearance rate (Ra) (C), and insulin-mediated glucose disappearance rate (∆Ra) (D) during euglycemic hyperinsulinemic clamp studies from time 0 to 120 min in fructose-fed hamsters treated with rosiglitazone (open circles, n = 5) versus placebo (closed circles, n = 6) versus hamsters fed a chow diet (control open squares) n = 5). Bars represent the mean ± S.E. Statistically significant differences between groups are indicated in the text below.](image-url)

the level of suppression of Ra from base line (Ra was suppressed to 64.7 ± 15.6 of base line versus 19.1 ± 11.4 versus 13.1 ± 8.4% of base line level during the clamp in the F, FR, and control chow-fed group respectively, p < 0.001 for the difference between F and the two other groups). The glucose infusion rate (not shown) was significantly lower in the F versus the FR group during the last 30 min of the clamp (64.7 ± 8.7 versus 121.7 ± 25.1 μmol/kg/min, p < 0.001). However, rosiglitazone treatment did not completely correct the glucose infusion rate and remained lower than the control chow-fed group (glucose infusion rate of control chow-fed group, 176.2 ± 3.0 μmol/kg/min, p < 0.001 versus FR group). Consequently, insulin-mediated glucose disappearance rate (∆Rd) (Fig. 1D) during the clamp was also significantly lower in the F versus FR animals (29.4 ± 8.4 versus 75.3 ± 20.8 μmol/kg/min, p < 0.001) but was not completely normalized by treatment with rosiglitazone (∆Rd of control chow-fed group, 119.6 ± 5.1 μmol/kg/min, p < 0.001 versus FR).

**Insulin Signaling in Hamster Primary Hepatocyte Cultures**—In hepatocytes isolated from F, insulin-stimulated insulin receptor β subunit tyrosine phosphorylation was reduced to 34.1 ± 2.6% (n = 3, p = 0.033) of that in control hepatocytes derived from chow-fed hamsters, and this was restored to the control levels (98.3 ± 5.3, n = 3, p = 0.01 versus F) after rosiglitazone treatment, indicating complete restoration of insulin receptor phosphorylation by the drug (Fig. 2A). Insulin
receptor appears as a doublet on the gel. We have consistently observed this doublet in hamster hepatocytes. We do not believe that the second band is a result of degradation, since the addition of protease inhibitors does not prevent the detection of the doublet (data not shown). Insulin-stimulated IRS-1 phosphorylation versus basal was $184.3 \pm 22.6\%$ in the control chow-fed ($n = 4, p = 0.002$), $130.3 \pm 5.3\%$ in F ($n = 4, p = 0.007$), and $188.9 \pm 5.8\%$ in FR ($n = 4, p = 0.001$) (Fig. 2B), indicating improvement of IRS-1 phosphorylation to the control levels in hepatocytes isolated from FR ($p < 0.001$ F versus FR and $p = 0.49$ for control chow-fed versus FR groups).

The effect of insulin on phosphorylation of IRS-2 was similar to that of IRS-1, as shown in Fig. 2C, indicating significant reduction ($n = 3, p = 0.01$ versus control chow-fed group) in insulin-stimulated IRS-2 phosphorylation with fructose feeding and a marked improvement ($n = 3, p = 0.004$ versus F) after treatment with rosiglitazone. As shown in Fig. 3A, fructose feeding had no significant effect on IR protein mass ($100 \pm 14.1\%$ in the control chow-fed group versus $88.3 \pm 29.8\%$ in F, $n = 4, p = 0.3$). However, in FR hepatocytes, IR protein mass was increased more than 2-fold versus cells derived from control chow-fed and F animals ($212.6 \pm 47\%$ of control chow-fed animals, $n = 4, p = 0.001$ versus F). Fructose feeding reduced the protein mass of IRS-1 (Fig. 3B) by $77\%$ from $359.7 \pm 23.9$ scanning units/mg of total protein in hepatocytes from control chow-fed animals to $80 \pm 11.5$ in hepatocytes from F animals ($n = 3, p = 0.0002$ control chow-fed versus F). Rosiglitazone treatment partially restored IRS-1 mass to $52.8 \pm 7.1\%$ that of control chow-fed animals ($n = 3, p = 0.003$ versus F).

IRS-2 protein mass in hepatocytes isolated from F hamsters was reduced to $57.8 \pm 7.1\%$ ($p = 0.001$) that of the levels in control chow-fed animals, whereas rosiglitazone treatment increased protein mass to $74.1 \pm 8\%$ that of control hepatocytes ($n = 4, p = 0.002$ versus F) (Fig. 3C). These data suggest that the observed change in IR, IRS-1, and IRS-2 phosphorylation in hepatocytes isolated from FR may be partially due to change in protein expression levels of these proteins.

Interestingly, PTP-1B protein mass increased to $169.9 \pm 13.2\%$ ($n = 3, p = 0.0002$) that of controls with fructose feeding. FR had marked reduction of PTP-1B levels to $24.4 \pm 12.9\%$ that of control chow-fed animals ($n = 3, p = 0.0004$ versus F) (Fig. 3D).
Treatment of Fructose-fed Hamsters with Rosiglitazone Ameliorates VLDL-apoB and VLDL-TG Oversecretion in Vivo and ex Vivo without Affecting VLDL Clearance

The slope of the increase in VLDL-apoB (Fig. 4A) over time after the injection of Triton WR-1339 was significantly steeper in the F versus FR group (2.42 ± 0.51 versus 1.09 ± 0.27 μg/ml/min, p < 0.05) and versus the control Chow-fed group (0.3 ± 0.1 μg/ml/min, p < 0.05). Consequently, the VLDL-apoB secretion rate was higher in the F versus FR group (12.4 ± 2.7 vs 5.5 ± 1.4 μg/min, respectively, p < 0.05) and versus the control Chow-fed group (1.3 ± 0.3 μg/min, p < 0.05) (inset of Fig. 4A). Similarly, VLDL-TG increase over time after the injection of Triton WR-1339 (Fig. 4B) was significantly higher in F versus FR hamsters (0.024 ± 0.004 versus 0.011 ± 0.004 μmol/ml/min, respectively, p < 0.05) and versus the control Chow-fed group (0.009 ± 0.002 μmol/ml/min, p < 0.05). The VLDL-TG secretion rate (inset of Fig. 4B) was higher in the F than in the FR group (0.12 ± 0.02 versus 0.06 ± 0.02 μmol/min respectively, p < 0.05) and higher than the control Chow-fed group (0.04 ± 0.01 μmol/min, p < 0.05). As depicted in Fig. 4C, rosiglitazone treatment significantly reduced ex vivo VLDL-apoB secretion to 38 ± 32% (mean ± S.D., n = 4, p < 0.001) that of fructose-fed hepatocytes, in keeping with the in vivo findings. In vivo VLDL-TG fractional clearance rate, as determined from the [2-3H]glycerol bolus studies, was not significantly different between the F versus FR animals (0.034 ± 0.008 versus 0.025 ± 0.004 min⁻¹ respectively, p = 0.33), although clearance tended to be slightly delayed in the latter.

Treatment of Fructose-fed Hamsters with Rosiglitazone Leads to Intracellular Destabilization of Nascent VLDL Particles and Correction of Enhanced Expression of MTP—In pulse-chase labeling experiments, after a 1-h chase, there was a significant reduction in the fraction of apoB secreted (Fig. 5A) in hepatocytes from F versus FR animals (88 ± 3% versus 49 ± 6% respectively, p = 0.001). Decreased secretion was also accompanied with a significant decrease in total apoB recovered (Fig. 5B). There was also a significant reduction in the fraction of labeled apoB secreted in the FR versus F animals after a 2-h chase (53 ± 7% versus 97 ± 1% in the FR versus F animals, respectively, p = 0.004), and similarly higher levels of total apoB were recovered, suggesting that rosiglitazone treatment led to destabilization and increased degradation of nascent apoB-containing particles. The cellular protein mass of MTP in hepatocytes from F was 153.3 ± 6.6% (n = 4, p = 0.0002) that of controls (Fig. 5C). Rosiglitazone treatment led to normalization of cellular protein mass of MTP in fructose-fed hamsters to 107.0 ± 9.4% that of controls (n = 4, p < 0.005 versus F).

Discussion

In the present study we have demonstrated that treatment of fructose-fed insulin-resistant hamsters with rosiglitazone, a member of the thiazolidinedione class of insulin sensitizers with specific peroxisome proliferator-activated receptor γ agonist activity, improved whole body and liver insulin sensitivity in vivo and insulin signaling in the liver and reduced VLDL secretion in vivo and ex vivo. Furthermore, rosiglitazone treatment was associated with a reversal of the increased expression of MTP seen with fructose feeding and with de-stabilization of intracellular nascent apoB-containing lipoproteins, indicating potential molecular mechanisms by which insulin sensitization led to reduction of VLDL secretion in this insulin-resistant animal model.

Treatment with rosiglitazone has been shown to improve glucose metabolism at least in part by improving skeletal mus-
versus placebo (28,800). Fructose-fed hamsters treated with rosiglitazone (c) in hepatocytes derived from fructose-fed hamsters treated with rosiglitazone (open bars, n = 4) versus placebo (closed bars, n = 3). Data are shown as the mean ± S.D.

The VLDL clearance rate was slightly lower with rosiglitazone treatment compared to placebo. This is consistent with the demonstration of increased whole-body glucose disposal rate with treatment of fructose-fed hamsters in the present study. Rosiglitazone treatment has also resulted in insulin sensitization of adipose tissue (20) and has often led to a reduction of plasma FFA levels and flux (21, 22). Although rosiglitazone treatment did not result in a significant reduction in fasting plasma FFA in the present study, we cannot rule out that rosiglitazone treatment in this model may have resulted in lower postprandial FFA levels and lower overall FFA flux to the liver. If this were the case, reduced FFA flux to the liver could have accounted in part for the reduced VLDL secretion with rosiglitazone treatment. More studies will be required to evaluate this possibility.

Reduction of TG secretion with thiazolidinedione treatment has also been found in sucrose-fed and obese Zucker rats by other investigators (22, 23). Nevertheless, most published studies in rats or mice did not show an inhibitory effect of thiazolidinediones on VLDL secretion, thereby concluding that the lowering of plasma TG resulted in total or in part from increased VLDL clearance (22, 24, 25). Unlike the fructose-fed hamster and insulin resistant humans, the rodent models used in the latter studies display impaired plasma TG clearance as the major mechanism of their hypertriglyceridemia when they become insulin-resistant (8). This perhaps explains the discrepancy between our results and those of the latter studies. Also, unlike the present study, previous studies did not directly assess VLDL clearance. Whether rosiglitazone and other thiazolidinediones can affect lipoprotein lipase expression and activity in animals and humans is controversial, with some studies showing increased expression and activity (24, 26) but others showing either no effect (27) or even reduced expression and activity in adipose tissue (28).

A limitation of the tritiated glycerol method used in the present study to assess VLDL clearance is that any change in de novo lipogenesis induced by treatment with rosiglitazone in the present study could result in a change in the relative contribution of glycerol-derived palmitate synthesis to VLDL-TG turnover, resulting in some error in the assessment of VLDL-TG glycerol turnover. To our knowledge, no previous study has addressed whether treatment with a thiazolidinedione results in alteration of fructose-induced elevation of in vivo hepatic de novo lipogenesis. Although a putative effect of rosiglitazone on the induction of hepatic de novo lipogenesis (29) may be expected to somewhat alter VLDL-TG glycerol turnover, de novo lipogenesis contributes less than 20% of total VLDL-TG turnover in fructose-fed rodents (30). Because only a fraction of hepatic de novo lipogenesis is derived from glycerol, it is unlikely that any effect of rosiglitazone on de novo lipogenesis would significantly alter total VLDL-TG glycerol turnover.

Treatment with thiazolidinediones has resulted in either no significant reduction or, at best, a modest lowering of plasma TGs in clinical trials in humans with insulin resistance and Type 2 diabetes (31), despite their documented insulin sensitizing effects (21, 32, 33). This is consistent with our observation that treatment with rosiglitazone resulted in a non-significant reduction in fasting plasma TG levels in the fructose-fed hamster, an animal model of mild hypertriglyceridemia associated with VLDL over-secretion. A marked reduction of plasma TG levels after treatment with rosiglitazone and other thiazolidinediones has been more consistently shown in various mouse and rat models of insulin resistance and type 2 diabetes, animal models that display a much more pronounced fasting hypertriglyceridemia than the one usually found in insulin-resistant humans (23, 34, 35) and in our hamster model. In the present study, the reduction of VLDL secretion in the fructose-fed hamster accounted for the reduction of plasma TG levels associated with rosiglitazone treatment, since VLDL-TG clearance was not different with rosiglitazone treatment. In fact, the VLDL clearance rate was slightly lower with rosiglitazone treat-
ment versus fructose alone, which could explain why the 50% reduction of VLDL secretion observed both in vivo and ex vivo with rosiglitazone treatment did not translate into a significant reduction in fasting plasma TG levels. To our knowledge, the effect of treatment with rosiglitazone on VLDL production and clearance in humans has not been reported.

In the present study, we documented definite improvement in the insulin-signaling cascade in hepatocytes isolated from fructose-fed hamsters treated with rosiglitazone as well as a significant reduction of endogenous glucose production in vivo. Whether the improved hepatic insulin sensitization in the present study resulted from a direct hepatic effect of rosiglitazone or from an indirect effect, secondary to the action of rosiglitazone on extrahepatic tissues, is unclear. We showed that primary hepatocytes from fructose-fed hamsters display a significant increase in PTP-1B expression, which was markedly reduced with rosiglitazone treatment. PTP-1B has been shown to dephosphorylate the insulin receptor and perhaps also IRS-1 and plays a very important role in the regulation of insulin signaling (36). Increased PTP-1B expression in skeletal muscle, adipose tissue, and liver has also been found in other animal models of insulin resistance and diabetes (37–39) and in humans with obesity or diabetes (40, 41). Knock-out mice for this enzyme are very sensitive to insulin, are resistant to fat-induced insulin resistance, and display an increased phosphorylation of liver and muscle insulin receptor after insulin injection (42, 43). We have recently shown that increased expression of PTP-1B precedes the reduction of insulin-mediated tyrosine phosphorylation of IRS-1 and IRS-2 observed in primary hamster hepatocytes with prolonged ex vivo exposure to high concentrations of insulin (44). We have also shown that incubation with vanadate, a general phosphatase inhibitor, leads to a dose-dependent reduction in cellular and secreted apoB (44), a finding that has also been reported in primary rat hepatocytes (45).

To our knowledge, this is the first report of the effect of treatment with a thiazolidinedione on PTP-1B expression. Clearly, this PTP-1B-lowering effect of rosiglitazone could be a very important potential mechanism for the liver insulin-sensitizing effect of this drug observed in our study.

**FIG. 5. Pulse-chase labeling experiments to assess the stability of apoB in hepatocytes from fructose-fed hamsters treated with rosiglitazone.** A, distribution of immunoprecipitated apoB in media (Secreted apoB). B, immunoprecipitable apoB remaining in cells + media (total apoB). The fructose-fed + rosiglitazone-treated (closed circles) versus fructose-fed + placebo-treated group (open circles) expressed as a percentage of radiolabeled apoB at time 0. *, significantly different from fructose-fed hepatocytes (secreted apoB; p = 0.001 at 1 h, p = 0.004 at 2 h). **, significantly different from fructose-fed hepatocytes (total apoB; p = 0.001 at 1 h, p = 0.0095 at 2 h) (n = 3). C, microsomal MTP expression. Data are shown for hepatocytes from control hamsters fed regular chow and from fructose-fed hamsters treated with rosiglitazone versus placebo as indicated (n = 4 per group, p < 0.005 for the difference between fructose-fed + rosiglitazone versus fructose-fed + placebo animals). The MTP bands were quantitated by densitometric scanning, and the mass of the 97-kDa MTP subunit detected was expressed as a percentage of the MTP mass detected in control cells. Please note that the blot shows the result of one representative experiment, whereas the graph displays the mean ± S.D. of four independent experiments. They are not therefore exactly the same. Data are the mean ± S.D.
Further studies are needed to address whether this occurs as a direct effect at the liver or secondary to changes induced in extra-hepatic tissues and whether these findings are specific to the fructose-fed hamster model or can be generalized to humans.

The reduction in MTP levels with rosiglitazone treatment may have been implicated in the reduction of VLDL secretion in the present study. MTP plays an important role in VLDL assembly and intracellular stabilization of apoB (46), although it may not be required for the late lipidation of the particle (47). The promoter region of the MTP gene contains a negative insulin-response element (48), and insulin, acting through its receptor, can lower MTP expression in HepG2 cells (49). Therefore, it is likely that the reduction in MTP levels induced by rosiglitazone treatment was a consequence of improvements in insulin signaling at the liver. However, the precise molecular-signaling pathway involved in insulin-mediated modulation of MTP expression is currently unclear. Given the complexity of insulin regulation of VLDL secretion, it is unlikely that modulation of MTP levels in the liver associated with insulin sensitization is the sole explanation for the rosiglitazone-induced reduction of intracellular apoB-containing particle stability and consequent VLDL secretion.

We have previously shown that fructose feeding results in increased apoB stability and VLDL assembly in the Syrian Golden hamster (11). An important finding in the present study was the reduction in nascent apoB stability with rosiglitazone treatment. We have recently shown that ~40% of nascent apoB is degraded intracellularly in hamster hepatocytes (10). Post-translational apoB degradation is felt to be an important regulatory mechanism controlling the rate of VLDL secretion (50). The factors regulating apoB degradation are complex, but hepatocyte lipid availability, insulin action, and MTP activity are three important factors (50). Rosiglitazone treatment could have reduced apoB stability in the fructose-fed hamster by any one of these mechanisms, i.e. by reducing FFA flux to the liver and, hence, reducing hepatocyte triglycerides, by improving insulin action and, hence, increasing apoB degradation, or by reducing MTP activity and, hence, reducing nascent VLDL particle assembly.

In conclusion, we have shown that whole-body and hepatic insulin sensitization with rosiglitazone treatment is associated with a reduction in hepatic MTP expression, apoB stability, and VLDL secretion in the fructose-fed insulin-resistant hamster. Our findings suggest that therapeutic measures that effectively ameliorate hepatic insulin sensitivity or that reduce MTP overexpression in insulin resistant states could be part of the strategy to correct the VLDL oversecretion associated with insulin resistance.

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