Troglitazone Ameliorates Abnormal Activity of Protein Tyrosine Phosphatase in Adipose Tissues of Otsuka Long-Evans Tokushima Fatty Rats

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Tagami, S., Honda, T., Yoshimura, H., Homma, H., Ohno, K., Ide, H., Sakaue, S. and Kawakami, Y. Troglitazone Ameliorates Abnormal Activity of Protein Tyrosine Phosphatase in Adipose Tissues of Otsuka Long-Evans Tokushima Fatty Rats. Tohoku J. Exp. Med., 2002, 197 (3), 169-181 —— Protein tyrosine phosphatases (PTPases) play an essential role in the regulation of steady-state phosphorylation of the insulin receptor and other proteins in the insulin signaling pathway. To determine the role of PTPases in adipose tissue in the development into an insulin-resistant state, we examined PTPase activities and protein levels of three major candidate PTPases in adipose tissues of 26-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Particulate PTPase activities in visceral and epididymal adipose tissues of OLETF rats were increased compared to those in Long-Evans Tokushima Otsuka (LETO) rats, non-insulin-resistant controls. Cytosolic PTPase activities in these tissues were conversely decreased in OLETF rats. In subcutaneous adipose tissues, those changes were not observed. Western blot analysis showed that the amounts of leukocyte antigen-related PTPase (LAR), PTPase 1B (PTP1B), and src homology 2-containing PTPase (SH-PTP2) were increased in particulate fractions of visceral and epididymal fat of OLETF rats. On the other hand, those in the cytosolic fractions were slightly decreased. Troglitazone was administered to OLETF rats to examine the effect of the drug on the changes in PTPase activity and distribution. Troglitazone treatment restored those alterations in PTPase activity in the particulate fraction and the amounts of LAR, PTP1B and SH-PTP2 in both fractions of visceral and epididymal adipose tissues of OLETF rats. Although it remains unknown whether such effects of troglitazone are mediated by peroxisome proliferator-activated receptor γ, these data provide useful information for under-

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Insulin resistance is a major feature of the pathophysiology of type 2 diabetes mellitus (Rabinowitz 1970; Kolterman et al. 1980; Caro 1991) and is also associated with a clustering of multiple risk factors of cardiovascular disease (DeFronzo and Ferrannini 1991). Since the prevalence of type 2 diabetes is increasing all over the world, the cause of insulin resistance must be elucidated as soon as possible. Although obesity is one of the factors leading to the state of insulin resistance, the mechanism by which obesity leads to this state is not known. It is now recognized that adipocytes play an important role in insulin resistance induced by obesity. Adipocyte-specific glucose transporter (GLUT) 4-knockout mouse expressed a phenotype of insulin resistance (Abel et al. 2001). This observation indicated the possibility that decline in insulin action of adipocytes alone causes insulin resistance of muscle and liver. Indeed, insulin signal transduction in adipose tissue of obese subjects with type 2 diabetes has been shown to be impaired (Friedenberg et al. 1987; Thies et al. 1990). Fat distribution also affects insulin sensitivity, which is more inhibited in patients with accumulation of visceral fat than in patients with accumulation of subcutaneous fat (Fujio et al. 1987). Those findings suggest that the metabolic effects of adipocytes are different depending on the region.

Tyrosine phosphorylations of the insulin receptor and its substrate proteins are early and pivotal events in insulin signal transduction (White and Kahn 1994). However, the molecular mechanisms involved in the regulation of these events are still not clear. Protein tyrosine phosphatase (PTPase) is required for dephosphorylation of proteins, including the insulin receptor and its substrates, and has the potential to regulate signals from the insulin receptor. Increased PTPase activity in subcutaneous adipose tissue of obese human subjects has been reported, and this may account for insulin resistance (Ahmad et al. 1995). However, there have been no reports of PTPase activity in visceral adipose tissue of obese subjects with insulin resistance. PTPases can be divided into several subtypes, some of which have been proposed to be regulators of the insulin action pathway; e.g., leukocyte antigen-related PTPase (LAR), PTPase 1B (PTP1B), and src homology 2-containing PTPase (SHP-2). Previous studies suggested that LAR and PTP1B regulate insulin signaling negatively in vitro (Känner et al. 1996; Li et al. 1996). Although SH-PTP2 seems to regulate insulin signaling positively, it may be less important for the development of insulin resistance induced by obesity (Ahmad et al. 1995; Haussdorff et al. 1995; Kharitonenkov et al. 1995; Chen et al. 1997). Increased insulin sensitivity and decreased adiposity in PTP1B-knockout mice indicate that PTPase is closely related to insulin action and body fat store (Eliechly et al. 1999; Klamann et al. 2000). However, it is still not clear whether PTPase of adipose tissue contributes to obesity-induced insulin resistance.

Troglitazone, a compound belonging to the family of thiazolidinediones (TZDs), has an antidiabetic effect (Kaneko 1997) by improving insulin resistance without increasing insulin secretion. It has been proposed that troglitazone elicits its effect by increasing the number of small adipocytes and decreasing the number
of large adipocytes in white adipose tissue (Okuno et al. 1998). That is, adipocytes are an important target for troglitazone. However, it is not known whether the drug has an effect on adipose PTPases.

In this study, we examined the activities and amounts of PTPases not only in subcutaneous adipose tissue but also in visceral and epididymal adipose tissues of an animal model of obesity-induced insulin resistance, Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Kawano et al. 1992). We also investigated the in vivo effects of troglitazone on the activities and amounts of PTPases in visceral and epididymal adipose tissues of OLETF rats. Our findings suggest that adipose PTPase, especially in visceral adipose tissue, plays an important role in the development of an insulin-resistant state and that the effect of troglitazone on insulin sensitivity is exerted through dynamic modulation of the alterations of PTPase activity in adipose tissue.

**MATERIALS AND METHODS**

**Materials**

[γ-32P]ATP was obtained from New England Nuclear (Boston, MA, USA). Troglitazone was a generous gift from Sankyo Co., Ltd. (Tokyo). Reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Dithiothreitol, phenylmethanesulfonyl fluoride (PMSF), leupeptin, benzamidine, pepstatin A, antipain, and aprotinin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Reagents, which were all of analytical grade, were purchased from Wako Pure Chemical (Osaka).

**Experimental animals**

Male nondiabetic control Long-Evans Tokushima Otsuka (LETO) and OLETF rats were raised at the Tokushima Research Institute (Tokushima). Twenty-six-week-old male LETO rats and age-matched male OLETF rats were used in all experiments. The rats were divided into groups of LETO rats, OLETF rats, troglitazone-treated LETO rats, and troglitazone-treated OLETF rats (n = 4 in each group). The animal studies conformed to the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

**Drug administration**

Troglitazone was administered to the rats for 7 days as 0.2% food admixtures. This concentration has been shown to elicit a hypoglycemic effect and not to cause any significant change in food intake (Fujiiwara et al. 1988, 1991). The administered dose of troglitazone, about 100 mg kg⁻¹ day⁻¹, was calculated from the food intake and body weight of each rat.

**Blood sampling and removal of adipose tissues**

For blood sampling, the tip of the tail of each rat was cut with a razor blade after 12 hours of fasting, and blood was collected in heparinized hematocrit tubes. The capillary tubes were centrifuged, and the separated plasma was collected in 0.5-ml microfuge tubes and frozen until use in assays. Each rat was sacrificed after 12 hours of fasting by decapitation, and adipose tissues were removed immediately.

**Analytical methods**

The plasma glucose level was determined using a glucose analyzer (Mitsubishi Chemical Co., Tokyo). Plasma triglyceride and total cholesterol levels were measured using commercial kits (Wako Pure Chemical). The plasma insulin level was measured by a modified radioimmunoassay micromethod as described previously (Fujiiwara et al. 1988).

**Tissue extracts and subcellular fractionation**

Fresh adipose tissues were homogenized on ice with a hand homogenizer in 3 volumes of cold buffer containing 50 mM Tris-HCl (pH
7.5), 0.25 M sucrose, 2 mM ethylenediaminetetraacetic acid, 2 mM ethylene glycol-bis (2-aminoethyl ether) tetraacetic acid, 7 mM 2-mercaptoethanol, 0.1 mM benzamidine, 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 6 μg/ml antipain (buffer A). The homogenates were centrifuged at 1000 g for 10 minutes at 4°C, and the resulting supernatants were used as crude extracts. The crude extracts were centrifuged at 105 000 g for 1 hour at 4°C, and the resulting supernatants were used as cytosolic fractions. The precipitates were rinsed 5 times with buffer A and then extracted with buffer A containing 5% (w/v) Nonidet P-40 (NP-40) (Sigma Chemical Co.). After centrifugation at 105 000 g for 1 hour at 4°C, the resulting supernatants were used as particulate fractions (expected to contain membrane-bound PTPases) (Matsuda et al. 1996).

Tyrosine phosphatase assay

Tyrosine phosphatase activity was measured in terms of the release of radiolabeled phosphate from the reduced carboxyamidemethylated lysozyme (RCM-lysozyme) as described previously (Swarup et al. 1983; Brunati et al. 1985; Tonks et al. 1988a, b). RCM-lysozyme was prepared by the procedure of Tonks et al. (1988a, b). The RCM-lysozyme was prepared by incubating RCM-lysozyme with rabbit spleen protein tyrosine kinase in a reaction mixture of 25 mM HEPES (pH 7.2), 25 mM MgCl2, 0.1 mM sodium vanadate, and 0.4 mM [γ-32P] ATP (400 cpm/pmol) for 6 hours at 30°C. The lysozyme was exclusively phosphorylated on tyrosine (>99%), and the phosphorylation rate was 0.3 mol/mol protein. The tyrosine kinase used in this reaction was purified from rabbit spleen as described previously (Swarup et al. 1983; Fujiwara et al. 1991). The reaction mixture for the PTP assay contained 25 mM imidazole-HCl (pH 7.2), 1 mg/ml bovine serum albumin (BSA), 0.5% NP-40, 280 mM 2-mercaptoethanol, 30 μM 32P-RCM-lysozyme, and tissue extract in a final volume of 60 μl. After 10 minutes at 30°C, the reaction was terminated with an equal volume of 20 mM silicotungstic acid in 0.01 M H2SO4. After 10 minutes on ice, the mixture was centrifuged at 12 500 g for 2 minutes. The supernatant was mixed with one-fifth volume of 5% ammonium molybdate in 2 M H2SO4 and extracted with an equal volume of isobutanol/benzene (1:1). The radioactivity in the upper phase was measured with a liquid scintillation counter. One unit (U) of PTP activity is defined as the enzyme activity that releases 1 nmol of phosphate per minute.

Western blot analysis

Western blot analysis was carried out essentially as described by Takizawa et al. (1994). Proteins (70 μg) of the particulate and cytosolic fractions were separated on a 7.5% SDS-PAGE gel under reducing conditions and electrophoretically transferred to a polyvinylidene difluoride membrane from Millipore Co. (Bedford, MA, USA). The membrane was blocked with phosphate-buffered saline containing 3% BSA (fatty acid-free, Sigma Chemical Co.) and 0.1% (v/v) Tween-20 and incubated with mouse anti-SH-PTP2, rabbit anti-PTP1B, or goat anti-LAR and then with 1 μg/ml horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG antibody (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, USA), HRP-conjugated donkey anti-rabbit IgG antibody (Chemicon International Inc., Temecula, CA, USA), or HRP-conjugated goat anti-IgG antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 1 hour. Immunoreactive bands were detected using an ECL Western blotting detection kit (Amersham International Plc, Buckinghamshire, United Kingdom). A monoclonal antibody to PTP1B and a polyclonal antibody to LAR were obtained from Transduction Laboratories (Lexington, KY, USA), and a polyclonal antibody to SH-PTP2.
was obtained from Santa Cruz Biotechnology Inc. Immunoreactive proteins were quantitated using a Fluor-S MAX MultiImager system (Bio-Rad Laboratories).

**Determination of protein concentration**

Protein concentration was measured by the DC Protein Assay (Bio-Rad Laboratories) using BSA as a standard.

**Statistical analysis**

All values are expressed as means ± s.e. Statistical significance was analyzed using analysis of variance followed by Sheffe’s test. A p-value of less than 0.05 was considered to be significant.

**RESULTS**

**Characteristics of the animals**

The body weights and the levels of fasting plasma glucose, insulin, total cholesterol, and triglyceride in the LETO, OLETF and troglitazone-treated rats that were used in the experiments are shown in Table 1. The mean body weight of the OLETF rats was significantly greater than that of the LEOTO rats at 26 weeks of age (p < 0.01). The plasma insulin level was higher in OLETF rats (p < 0.05), although there was no reduction in the plasma glucose level after 12-hour fasting. These findings indicated that the 26-week-old OLETF rats used in the present study had insulin resistance as described previously (Ishida et al. 1995). The fasting plasma glucose levels of OLETF rats were minimally increased. The OLETF rats also had significantly increased plasma triglyceride levels (p < 0.001), one of the characteristics of insulin resistance. Troglitazone treatment resulted in significant reductions in plasma triglyceride levels. Treatment with troglitazone tended to decrease plasma insulin and glucose levels but not significantly.

**PTPase activities in LEOTO, OLETF and troglitazone-treated rats**

PTPase activity was assessed with RCM-lysozyme as a substrate (Fig. 1). Particulate PTPase activities of the visceral and epididymal adipose tissues were increased in OLETF rats to levels 5.3-fold and 1.9-fold higher than the levels in control rats (visceral adipose tissue, 323.0 ± 9.7 vs. 60.5 ± 4.8 U/mg protein; epididymal adipose tissue, 158.5 ± 10.2 vs. 81.8 ± 3.0 U/mg protein). However, cytosolic PTPase activities in these adipose tissues of OLETF rats were conversely decreased by 83% and 29%, respectively (visceral adipose tissue, 57.0 ± 5.2 vs. 339.5 ± 12.9 U/mg

| Table 1: Characteristics of the animals used in the present study |
|------------------|----------------|----------------|----------------|----------------|
| BW (g) | FPG (mg/100 mL) | IRI (μU/mL) | TC (mg/100 mL) | TG (mg/100 mL) |
| **LETO rats** | | | | |
| No treatment (n=4) | 495.0 ± 22.1 | 144.8 ± 9.8 | 3.9 ± 0.5 | 82.5 ± 2.7 | 22.3 ± 2.7 |
| Troglitazone (n=4) | 513.8 ± 6.5 | 135.5 ± 4.5 | 4.0 ± 0.3 | 85.0 ± 2.0 | 23.0 ± 0.8 |
| **OLETF rats** | | | | |
| No treatment (n=4) | 628.8 ± 19.0** | 191.5 ± 17.8 | 10.2 ± 1.2* | 91.3 ± 3.6 | 124.8 ± 11.6*** |
| Troglitazone (n=4) | 616.3 ± 22.5 | 169.5 ± 13.8 | 6.1 ± 2.0 | 78.5 ± 3.4 | 61.0 ± 15.2** |

BW, body weight; FPG, fasting plasma glucose; IRI, immunoreactive insulin; TC, total cholesterol; TG, triglyceride; LEOTO, Long-Evans Tokushima Otsuka; Toroglitzazone, rats treated with troglitazone; OLETF, Otsuka Long-Evans Tokushima Fatty. Values are mean ± s.e. *p < 0.05 vs. not treated LEOTO rat group; **p < 0.01 vs. not treated LEOTO rat group; ***p < 0.001 vs. not treated LEOTO rat group; ?p < 0.01 vs. not treated OLETF rat group.
protein; epididymal adipose tissue, 114.7±8.9 vs. 162.1±12.1 U/mg protein). Despite these obvious changes in visceral and epididymal fat, particulate and cytosolic PTPase activities of the subcutaneous adipose tissues were not changed (data not shown). Troglitazone was also administered to the rats to examine the effect of troglitazone on PTPase. Troglitazone treatment to OLETF rats considerably restored the alterations of the particulate PTPase activities (visceral adipose tissue, 122.5±3.4 U/mg protein; epididymal adipose tissue, 110.7±5.7 U/mg protein) but had no effect on cytosolic PTPase (visceral adipose tissue, 92.5±4.8 U/mg protein; epididymal adipose tissue, 120.6±5.1 U/mg protein).

**Western blot analysis of LAR, PTP1B and SH-PTP2**

To determine the reasons for the changes in PTPase activities mentioned above, we analyzed the protein level of each candidate PTPase, LAR, PTP1B and SH-PTP2, associated with insulin action using Western blotting. As shown in Fig.2 (A) and Fig.3 (A), the amounts of LAR, PTP1B and SH-PTP2 in the particulate fractions in visceral and epididymal adipose tissues of OLETF rats were
significantly increased (to levels of 317%, 472%, and 184% for LAR, PTP1B and SH-PTP2, respectively, in visceral adipose tissue and 239%, 136% and 234% for LAR, PTP1B and SH-PTP2, respectively, in epididymal adipose tissue of those in LETO rats). The increased content of each PTPase in the particulate fraction was restored by troglitazone treatment (to levels of 112%, 119% and 127% for LAR, PTP1B and SH-PTP2, respectively, in visceral adipose tissue and 125%, 119% and 118% for LAR, PTP1B and SH-PTP2, respectively, in epididymal adipose tissue of those in LETO rats). Those findings were concordant with the results of PTPase activities. On the other hand, the amounts of PTP1B and SH-PTP2 in OLETF cytosolic fractions were slightly decreased as shown in Fig. 2 (B) and Fig. 3 (B) (to 86% and 80% for PTP1B and SH-PTP2, respectively, in visceral adipose tissue and 74% and 81% for PTP1B and SH-PTP2, respectively, in epididymal adipose tissue of those in LETO rats).

(A) Particulate fraction

LAR  
PTP1B  
SH-PTP2  
Troglitazone  
LETO  
OLETF

(B) Cytosolic fraction

PTP1B  
SH-PTP2  
Troglitazone  
LETO  
OLETF

Fig. 2. Western blot analysis of LAR, PTP1B, and SH-PTP2 in particulate (A) and cytosolic (B) fractions in visceral adipose tissues. Visceral adipose tissues were separated into particulate and cytosolic fractions. (A) Particulate fractions containing 70 μg protein were subjected to Western blot analysis with anti-LAR, anti-PTP1B or anti-SH-PTP2 antibodies. (B) Cytosolic fractions containing 70 μg protein were subjected to Western blot analysis with anti-PTP1B or anti-SH-PTP2 antibodies. The bands corresponding to LAR, PTP1B and SH-PTP2 are indicated by arrows.
LET0 rats). The contents of PTP1B and PH-PTP2 in cytosolic fractions were increased by troglitazone treatment (to 149% and 258% for PTP1B and SH-PTP2, respectively, in visceral adipose tissue and 82% and 219% for PTP1B and SH-PTP2, respectively, in epididymal adipose tissue of those in LET0 rats).

**DISCUSSION**

We demonstrated increased PTPase activities in particulate fractions and decreased PTPase activities in cytosolic fractions of visceral and epididymal adipose tissues of OLETF rats, an animal model of obesity-induced insulin resistance. Those changes in particulate PTPase activities paralleled changes in protein levels of LAR, PTP1B and SH-PTP2, candidate PTPases for the regulation of insulin action. However, those changes were not observed in subcutaneous adipose tissues. This is the first study to show such changes in PTPase activities.

![Western blot analysis](image)

**Fig. 3.** Western blot analysis of LAR, PTP1B, and SH-PTP2 in particulate (A) and cytosolic (B) fractions in epididymal adipose tissues. Epididymal adipose tissues were separated into particulate and cytosolic fractions. (A) Particulate fractions containing 70 μg protein were subjected to Western blot analysis with anti-LAR, anti-PTP1B or anti-SH-PTP2 antibodies. (B) Cytosolic fractions containing 70 μg protein were subjected to Western blot analysis with anti-PTP1B or anti-SH-PTP2 antibodies. The bands corresponding to LAR, PTP1B and SH-PTP2 are indicated by arrows.
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in fractionated visceral and epididymal adipose tissues of insulin-resistant rodents with obesity, though PTPase activities have been investigated in homogenates of subcutaneous adipose tissues of obese subjects and in livers and muscles of insulin-resistant rodents (Ahmad et al. 1995; Ahmad and Goldstein 1995; Pugazhenthii et al. 1995). We also demonstrated that troglitazone treatment restored those changes in activities and amounts of PTPase in vivo except for PTPase activity in the cytosolic fraction. This is also the first study to show a relationship between adipose PTPase and troglitazone.

It was previously demonstrated that PTPase activity was increased in homogenates of subcutaneous adipose tissue from obese subjects and that the activity had a strong positive correlation with body mass index (Ahmad et al. 1995). Furthermore, it was suggested that increases in the amounts of LAR and PTP1B proteins affect insulin sensitivity of obese subjects (Ahmad et al. 1995, 1997b). However, another study, in which subcutaneous adipose PTPase was examined, demonstrated that PTP1B activities were decreased in obese subjects despite an increase in PTP1B protein levels (Cheung et al. 1999). Given those conflicting results, the relationship between adipose PTPase and insulin resistance is not clear.

Our results concerning subcutaneous adipose PTPase activities in OLETF rats are also not consistent with previously reported data. One reason may be the difference in substrates used in measurements of PTPase activities. In this study, we used 26-week-old OLETF rats that had hyperinsulinemia with slight increase in fasting plasma glucose, suggesting that the rats had insulin resistance. Based on previous reports, the rats might have had an early stage of diabetes in which only the feeding plasma glucose levels were increased (Kawano et al. 1992), although we could not determine the plasma glucose levels after glucose loading. The difference between our results and those of previous studies might have been caused by the difference in degrees of glucose tolerance as Ahmad et al. (1997a) had shown in PTPase activity of muscle.

The fact that there was no difference between PTPase activities in subcutaneous adipose tissues in OLETF and LETO rats appears to be due to no relationship between adipose PTPase and insulin sensitivity. However, unlike in previous studies, we examined PTPase activities not only in subcutaneous adipose tissue but also in visceral and epididymal adipose tissues, and we also examined PTPase activities in fractionated samples of each adipose tissue of insulin-resistant rats with obesity. Fat distribution, particularly the degree of visceral fat accumulation, greatly contributes to insulin resistance (Fujioka et al. 1987). Visceral adipocytes have lower sensitivity to the antilipolytic effects of insulin than do subcutaneous adipocytes (Bolinder et al. 1983). Subsequently, accumulation of visceral fat facilitates an increase in free fatty acid levels, which reduces insulin sensitivity in the liver and muscle (Shulman 2000). Those findings suggest that visceral fat is more closely associated with insulin sensitivity than is subcutaneous fat. A difference between PTPase activities in subcutaneous and visceral adipose tissues has also been found in non-diabetic humans, and this difference might account for differential insulin sensitivity (Wu et al. 2001). We therefore believe that alteration in PTPase activity in visceral adipose tissue is more important for the development of insulin resistance. We did not examine tyrosine phosphorylation of the insulin receptor and the insulin receptor substrate (IRS)-1 in adipose tissue of the OLETF rat. However, the increased PTPase activity in the particulate fraction may, at least in part, contribute to the development of a state of insulin resistance in OLETF rats, since it has been shown that PTPase can dephosphorylate the insulin receptor or the IRS-1 in the particu-
late fraction of adipocytes (Kublaoui et al. 1995; Calera et al. 2000). Interestingly, PTPase activities in the cytosolic fraction were, conversely, decreased in adipose tissues of OLETF rats. Although the reason for this is not known, this finding suggests that there is a relationship between cytosolic PTPase and obesity.

We showed that protein levels of LAR, PTP1B and SH-PTP2 changed in parallel with changes in PTPase activities in the particulate fraction. The alterations in PTPase activities may be partly due to changes in those protein levels. Increased amounts of LAR and PTP1B in homogenates of subcutaneous adipose tissues of obese and diabetic subjects have been consistently shown, although the results of PTPase activities have been controversial (Ahmad et al. 1995; Cheung et al. 1999). Our results regarding the protein levels in particulate fractions agree with the results of those studies. Taken together, the results suggest that the increased abundance of LAR and PTP1B is linked between adiposity and insulin sensitivity. The findings of slight decreases in protein levels of PTP1B and SH-PTP2 in the cytosolic fraction were the opposite to those in the particulate fraction. Those findings suggest that there is an abnormal intracellular distribution of each PTPase in adipocytes of diabetic rats with obesity. However, the magnitude of decrease in protein levels seemed to be smaller than that in PTPase activity in visceral adipose tissue. There may be some factors other than protein levels contributing to PTPase activity.

In this study, troglitazone ameliorated abnormal PTPase activities and amounts of LAR, PTP1B and SH-PTP2 in the particulate fractions of visceral and epididymal adipose tissues. Because PTPases, particularly LAR and PTP1B, negatively regulate insulin signaling, decreases in the activities and amounts of PTPases in the particulate fraction of adipose tissue might result in an improvement in insulin resistance. TZDs, including troglitazone, are ligands for peroxisome proliferator-activated receptor (PPARγ) (Lehmann et al. 1995), which is a transcription factor expressed mainly in adipocytes (Tontonoz et al. 1994). It has been proposed that TZDs exert their effects by activating PPARγ, because there was a correlation between the hypoglycemic action of TZDs and their affinity for PPARγ (Berger et al. 1996; Willson et al. 1996). PPARγ might also mediate the effects of troglitazone on PTPases observed in this study. However, this issue has not been clarified in the present study. Further studies using other TZDs are needed to elucidate the mechanism underlying the effects of TZDs.

It should be pointed out that our results can not rule out the possibility that another action of troglitazone, such as an augmentation of insulin action, secondarily normalized PTPase activity and abundance. However, even if the decrease in PTPase activity in the particulate fraction was caused by an indirect effect of troglitazone, it must further increase insulin sensitivity. Troglitazone increased the amounts of PTP1B and SH-PTP2 but not PTPase activity in the cytosolic fraction, suggesting that cytosolic PTPase activity was not determined only by the protein levels and that the effects of troglitazone were not related to cytosolic PTPase activity.

In conclusion, an increase in PTPase activities in particulate fractions of visceral and epididymal adipose tissues may influence the insulin resistance induced by obesity more than does an increase in PTPase activities in subcutaneous adipose tissue. Troglitazone may improve insulin resistance via the normalization of PTPase activity in vivo. Further in vitro studies are needed to determine the effects of troglitazone on PTPases.

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References


