

## Full-length article

**Antidiabetic effect of a novel non-thiazolidinedione PPAR  $\gamma/\alpha$  agonist on *ob/ob* mice<sup>1</sup>**Xi HU, Ying FENG, Yu SHEN, Xiao-feng ZHAO, Juan-hong YU, Yu-she YANG, Ying LENG<sup>2</sup>

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**Key words**

diabetes; insulin-sensitizing; peroxisome proliferator-activated receptors; thiazolidinediones

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**Abstract**

**Aim:** To study whether T33, a new synthesized non-thiazolidinedione (TZD) peroxisome proliferator-activated receptor (PPAR)  $\gamma/\alpha$  dual agonist has an antidiabetic effect on *ob/ob* mice. **Methods:** *Ob/ob* mice were treated with 4 mg/kg or 8 mg/kg T33 by gavage for 20 d. Blood glucose levels were measured regularly. An oral glucose tolerance test (OGTT) and an insulin tolerance test (ITT) were performed on d 8 and d 12, respectively. The levels of insulin, triglyceride and free fatty acid (FFA) in the serum were measured at the end of administration. The intramuscular and liver triglyceride content was also determined. **Results:** T33 reduced the hyperglycemia, hyperinsulinemia and hyperlipidemia of the *ob/ob* mice. The OGTT and ITT showed that the insulin resistance state of the *ob/ob* mice was obviously ameliorated after T33 treatment. After 20 d treatment with 8 mg/kg T33, the triglyceride content in the gastrocnemius muscle decreased significantly. T33 did not have any effect on triglyceride content in the liver, whereas rosiglitazone significantly increased the hepatocyte lipid deposition. **Conclusion:** The PPAR $\gamma/\alpha$  dual agonist T33 has antidiabetic and insulin-sensitizing effects in *ob/ob* mice. It has the potential to be a new therapeutic candidate for the treatment of type 2 diabetes.

**Introduction**

Non-insulin-dependent diabetes mellitus (NIDDM, type 2 diabetes) is characterized by abnormal carbohydrate, fat and protein metabolisms, which are attributed to the diminished production of insulin or defective insulin action (insulin resistance)<sup>[1]</sup>. Insulin resistance is characterized by the impairment in insulin-regulated metabolic actions, including glucose transport, glycogen synthesis and gene expression characteristics<sup>[2]</sup>. Insulin resistance is a key factor in the onset and progress of type 2 diabetes. Ameliorating insulin resistance is an important strategy in the development of new pharmacological treatment for type 2 diabetes.

The peroxisome proliferator-activated receptors (PPAR) are ligand-activated nuclear receptors that regulate the expression of genes related to the carbohydrate, lipid and protein metabolisms<sup>[1,3]</sup>. Up to now, 3 PPAR subtypes have been discovered and characterized (PPAR- $\alpha$ ,  $\delta$  and  $\gamma$ )<sup>[4]</sup>. Different PPAR subtypes have been shown to play different but crucial roles in some important diseases<sup>[4]</sup>. PPAR $\gamma$  is

expressed mainly in adipose tissues and plays a central role in adipogenesis and glucose homeostasis<sup>[3,5]</sup>. PPAR $\alpha$  is highly expressed in the liver, heart and the skeletal muscle and contributes to lipid metabolism. The activation of PPAR $\gamma$  and PPAR $\alpha$  modulates the expression of genes associated with carbohydrate, lipid and protein metabolisms, which in turn influence glucose uptake, insulin sensitivity and lipid metabolism<sup>[1,3]</sup>. Thus, because of the central role of PPAR isoforms in metabolisms, they have become attractive targets for drug discovery aimed at improving insulin sensitivity<sup>[6-9]</sup>. Thiazolidinediones (TZD) are a new class of oral antidiabetic agents that improve insulin sensitivity, lipid and glucose homeostasis in type 2 diabetes through the activation of PPAR $\gamma$ <sup>[10]</sup>. There are currently 2 available TZD for clinical treatment: rosiglitazone and pioglitazone, with a third earlier compound, troglitazone, withdrawn due to hepatotoxicity. TZD improve glucose homeostasis by increasing insulin sensitivity in peripheral tissues<sup>[11-13]</sup>. The activation of PPAR $\alpha$  lowers triglycerides and elevates plasma HDL cholesterol levels<sup>[14,15]</sup>. The lipid modulating activities of fibrates

are presumably due to the activation of PPAR $\alpha$ . Since type 2 diabetic patients often develop hyperglycemia, insulin resistance, dyslipidemia and other metabolic abnormalities, dual PPAR $\gamma/\alpha$  agonist should be more beneficial in ameliorating major metabolic disorders than either PPAR $\alpha$  or PPAR $\gamma$  selective agonists.

The benzopyran derivative T33, originally named T11, is a novel non-thiazolidinedione agent. Our previous study reported its structure and showed that T33 could stimulate the differentiation of 3T3-L1 adipocytes and increase the insulin-induced triglyceride accumulation<sup>[16]</sup>. Using a cell-based reporter gene assay, T33 was identified as a PPAR $\gamma/\alpha$  dual agonist, which activated human PPAR $\gamma$  and PPAR $\alpha$  with EC<sub>50</sub> value of 19 and 148 nmol/L (unpublished data). Therefore, in the present study, the antidiabetic and insulin-sensitizing effects of T33 were evaluated in *ob/ob* mice, a type 2 diabetic animal model that were obese and insulin resistant.

## Materials and methods

**Compounds** T33 and rosiglitazone were synthesized by Prof Yu-she YANG of the Shanghai Institute of Materia Medica. Both compounds were prepared as suspensions in 1% carboxymethylcellulose solution for *in vivo* studies.

**Animals and treatment** The *ob/ob* mice and their lean control (6–7 weeks old), obtained from Jackson Laboratories (Bar Harbor, Maine, USA), were maintained under a 12:12 light-dark cycle with free access to water and food. After 2 weeks of acclimation, the *ob/ob* mice were weighed and bled via the tail vein after 5 h fasting for the blood glucose values test. The *ob/ob* mice were assigned to 4 groups based on fasting blood glucose values (first criterion) and initial body weight (second criterion). The *ob/ob* mice were gavaged once daily with vehicle (1% CMC), T33 (4 mg/kg), T33 (8 mg/kg) or rosiglitazone (4 mg/kg), respectively for 20 d. At the same time, the lean mice were also treated with vehicle (1% CMC). Blood glucose levels were tested regularly for the mice that were fed (tested at 9:00 AM) and fasted (tested at 14:00 PM after 5 h fasting) using a One-Touch Basic Glucose Monitor (Lifescan, Milpitas, CA, USA). Body weight was also measured regularly. All animal experiments were approved by the Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

**Oral glucose tolerance test** After 8 d treatment with the compounds, the *ob/ob* mice were subjected to an oral glucose tolerance test (OGTT). Briefly, the *ob/ob* mice were made to fast for 5 h and then orally administered with glucose (2.5 g/kg body weight). The blood glucose values were measured via blood drops obtained by clipping the tail of

the mice at 0, 30, 60, 120 min after glucose administration. The results of the OGTT were also expressed as integrated areas under the curves (AUC) over 120 min.

**Insulin tolerance test** After 12 d treatment with the compounds, insulin sensitivity was determined by performing an insulin tolerance test (ITT). After 5 h fasting, the mice were injected with biosynthetic human insulin (Novolin R; Novo Nordisk AIS, Bagsvaerd, Denmark) at 0.4 U/kg body weight subcutaneously, and blood glucose values were measured via blood drops obtained by clipping the tail of the mice at 0, 15, 40, 90, 120 and 240 min after the insulin injection. The results of the ITT were also expressed as the percentage of the reduction of blood glucose value.

**Blood sample analysis** After 20 d treatment with the compounds, blood samples were collected via retro-orbital sinus in the fed and fasted mice, respectively. The samples were separated into serum, immediately frozen at -20 °C, and stored until measurement. Serum insulin, triglyceride and free fatty acid (FFA) values were measured using the Insulin RIA Kit (Shanghai Institute of Biological Products, Shanghai, China), Triglycerides Kit (Shanghai Institute of Biological Products, Shanghai, China) and NEFA Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) respectively.

**Skeletal muscle and liver triglyceride measurement** After 20 d treatment with the compounds, the mice that had fasted for 5 h were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight). The gastrocnemius muscle and liver were removed and immediately frozen in liquid nitrogen, and then stored at -80 °C. The frozen skeletal muscle 15–20 mg or frozen liver 3–5 mg were used for triglyceride extraction. Each frozen tissue was added to 0.3 mL heptane-isopropanol-tween mixture (3:2:0.01 by volume) and homogenized. This homogenate was centrifuged at 1500×g at 4 °C for 15 min. Supernatants (upper phase contained extracted triglycerides) were collected and evaporated with vacuum centrifuge. The triglyceride content was determined using a triglyceride kit. All samples were measured in duplicates.

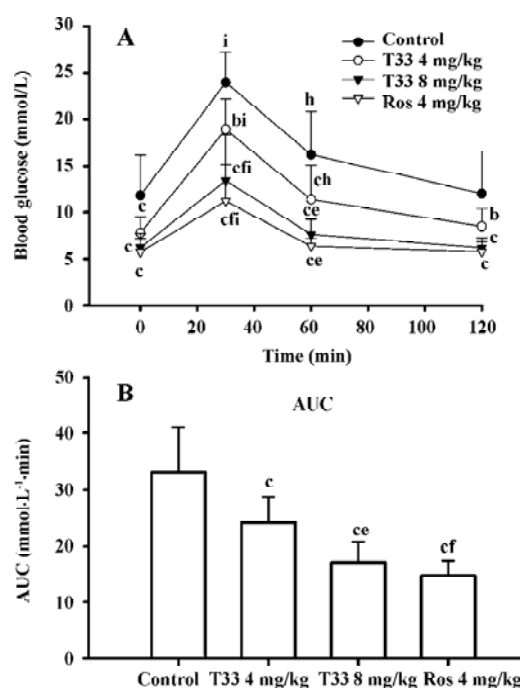
**Statistics** Results are expressed as mean±SD. Differences between groups were determined by one-way ANOVA. Fisher's least significant differences *post hoc* analysis was used to identify significant differences ( $P<0.05$ ). Differences between all time points with 0 min were analyzed by repeated ANOVA measurements in the OGTT and ITT experiments.

## Results

**Hypoglycemic effect of T33 on *ob/ob* mice** The blood

glucose level in the diabetic *ob/ob* mice was significantly higher than that of the lean mice. The *ob/ob* mice were treated with 4 mg/kg T33, 8 mg/kg T33, 4 mg/kg rosiglitazone or vehicle (1% CMC) for 20 d. After 4 d treatment, the significant decrease of the blood glucose level was observed in both the T33 and rosiglitazone-treated groups. T33 treatment led to dose-dependent reduction in blood glucose levels. The blood glucose levels of the mice that were fed and fasted decreased by 43.2% and 34.4%, respectively after 4 d treatment with 4 mg/kg T33, and the decrease rate of the 8 mg/kg T33-treated group was 57.6% and 39.7%, respectively (Tables 1, 2). During the 20 d treatment, the blood glucose levels of the 8 mg/kg T33-treated *ob/ob* mice that were fed and fasted were maintained at 4.5 to 7 mmol/L, which suggests that T33 could normalize the blood glucose of diabetic *ob/ob* mice. The hypoglycemic effect of T33 at a dose of 8 mg/kg was similar with that of rosiglitazone at a dose of 4 mg/kg on diabetic *ob/ob* mice (Tables 1, 2).

**Insulin-sensitizing effects of T33 on *ob/ob* mice** In the present study, the whole body insulin sensitivity was determined by performing OGTT and ITT. After 8 d treatment with the compounds, OGTT was performed. The *ob/ob* mice of the control group displayed a significantly stronger hyperglycemic response to an oral glucose administration, whereas the blood glucose level at each time point in both the 4 mg/kg and 8 mg/kg T33-treated *ob/ob* mice was lower than that of vehicle control (Figure 1A). The AUC in the



**Figure 1.** Effects of T33 on OGTT in *ob/ob* mice. *Ob/ob* mice were treated with T33 (4 mg/kg, 8 mg/kg), rosiglitazone (4 mg/kg) or vehicle (1% CMC) by gavage once daily for 20 d. OGTT was performed on d 8 of treatment (A), and the AUC of OGTT was calculated (B).  $n=8-12$ . Mean $\pm$ SD. <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs control group of *ob/ob* mice. <sup>e</sup> $P<0.05$ , <sup>f</sup> $P<0.01$  vs 4 mg/kg T33 group of *ob/ob* mice. <sup>h</sup> $P<0.05$ , <sup>i</sup> $P<0.01$  vs 0 min of each group.

**Table 1.** Effects of T33 on blood glucose values (mmol/L) in fed mice.  $n=8-12$ . Mean $\pm$ SD. <sup>c</sup> $P<0.01$  vs control group of *ob/ob* mice.

Group	Dose(mg/kg)	d 0	d 4	d 8	d 12	d 16
Lean	-	6.05 $\pm$ 0.49 <sup>c</sup>	5.51 $\pm$ 0.42 <sup>c</sup>	5.76 $\pm$ 0.65 <sup>c</sup>	5.78 $\pm$ 0.38 <sup>c</sup>	5.78 $\pm$ 0.55 <sup>c</sup>
Control	-	12.21 $\pm$ 4.06	14.12 $\pm$ 3.49	12.87 $\pm$ 3.84	14.49 $\pm$ 5.24	13.34 $\pm$ 5.44
T33	4	10.98 $\pm$ 4.01	8.01 $\pm$ 4.29 <sup>c</sup>	7.40 $\pm$ 2.73 <sup>c</sup>	7.83 $\pm$ 3.29 <sup>c</sup>	6.56 $\pm$ 1.42 <sup>c</sup>
T33	8	10.01 $\pm$ 3.90	5.99 $\pm$ 0.79 <sup>c</sup>	5.49 $\pm$ 0.91 <sup>c</sup>	5.38 $\pm$ 1.25 <sup>c</sup>	5.34 $\pm$ 1.02 <sup>c</sup>
Ros	4	10.62 $\pm$ 1.91	6.35 $\pm$ 0.84 <sup>c</sup>	5.30 $\pm$ 1.60 <sup>c</sup>	4.98 $\pm$ 0.73 <sup>c</sup>	5.52 $\pm$ 1.11 <sup>c</sup>

**Table 2.** Effects of T33 on blood glucose values (mmol/L) in fasted mice.  $n=8-12$ . Mean $\pm$ SD. <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs control group of *ob/ob* mice.

Group	Dose(mg/kg)	d 0	d 4	d 8	d 12	d 16
Lean	-	5.06 $\pm$ 0.66 <sup>c</sup>	5.25 $\pm$ 0.37 <sup>c</sup>	5.36 $\pm$ 0.51 <sup>c</sup>	5.29 $\pm$ 0.38 <sup>c</sup>	5.38 $\pm$ 0.27 <sup>c</sup>
Control	-	9.56 $\pm$ 2.90	10.39 $\pm$ 3.44	11.25 $\pm$ 4.11	12.91 $\pm$ 5.43	12.64 $\pm$ 6.75
T33	4	10.04 $\pm$ 2.44	6.81 $\pm$ 1.72 <sup>c</sup>	7.46 $\pm$ 1.76 <sup>c</sup>	7.13 $\pm$ 0.86 <sup>c</sup>	6.50 $\pm$ 1.08 <sup>c</sup>
T33	8	10.10 $\pm$ 2.50	6.27 $\pm$ 1.07 <sup>c</sup>	5.97 $\pm$ 1.37 <sup>c</sup>	5.30 $\pm$ 1.30 <sup>c</sup>	6.23 $\pm$ 1.03 <sup>b</sup>
Ros	4	9.33 $\pm$ 1.78	5.65 $\pm$ 0.98 <sup>c</sup>	5.75 $\pm$ 1.40 <sup>c</sup>	5.17 $\pm$ 0.73 <sup>c</sup>	6.00 $\pm$ 1.00 <sup>c</sup>

T33-treated group also significantly decreased (Figure 1B). Moreover, the improvement of T33 on the impaired oral glucose tolerance on *ob/ob* mice was dose-dependent. Rosiglitazone also improved the impaired oral glucose tolerance of *ob/ob* mice.

After 12 d treatment with T33 or rosiglitazone, ITT was performed in the *ob/ob* mice. No significant reduction of blood glucose level could be observed 15 min after the administration of insulin in the *ob/ob* mice of the vehicle control group, and only a 18.3% reduction could be observed at 40 min (Table 3), which suggests that *ob/ob* mice are insulin resistant. T33 at a dose of 4 mg/kg did not show any improvement of insulin tolerance, whereas the higher dose of T33 obviously improved insulin tolerance in the *ob/ob* mice. In the 8 mg/kg T33-treated group, the blood glucose value reduced by 28.9% and 44.5%, respectively at 15 and 40 min after the insulin injection, which suggests that T33 has a significant insulin sensitizing effect on *ob/ob* mice. Rosiglitazone 4 mg/kg also showed potential effects in the ITT, which is similar with that of T33 at a dose of 8 mg/kg.

**Effects of T33 on serum insulin, triglyceride, FFA and body weight of *ob/ob* mice** *Ob/ob* mice are characterized by advanced hyperinsulinemia. Our data shows that the serum insulin concentration of the mice that were both fed and fasted was significantly higher than that of the lean mice (Figure 2A). Treatment with T33 at a dose of 8 mg/kg for 20 d decreased serum insulin levels significantly in the fasted mice ( $P<0.01$ ), whereas no obvious reduction could be

observed in the *ob/ob* mice treated by T33 at a dose of 4 mg/kg. Rosiglitazone treatment also led to a significant ( $P<0.05$ ) reduction in serum insulin concentration in the fasted mice, whereas the reduction in the fed mice did not reach statistical difference.

T33 treatment at a dose of 4 mg/kg and 8 mg/kg resulted in a 37% and 48% reduction in serum triglyceride concentration in the fed mice ( $P<0.05$  and  $P<0.01$  vs vehicle control mice respectively), whereas no significant reduction could be observed in the fasted mice (Figure 2B). Rosiglitazone treatment also reduced serum triglyceride level in the fed mice, but not in the fasted mice.

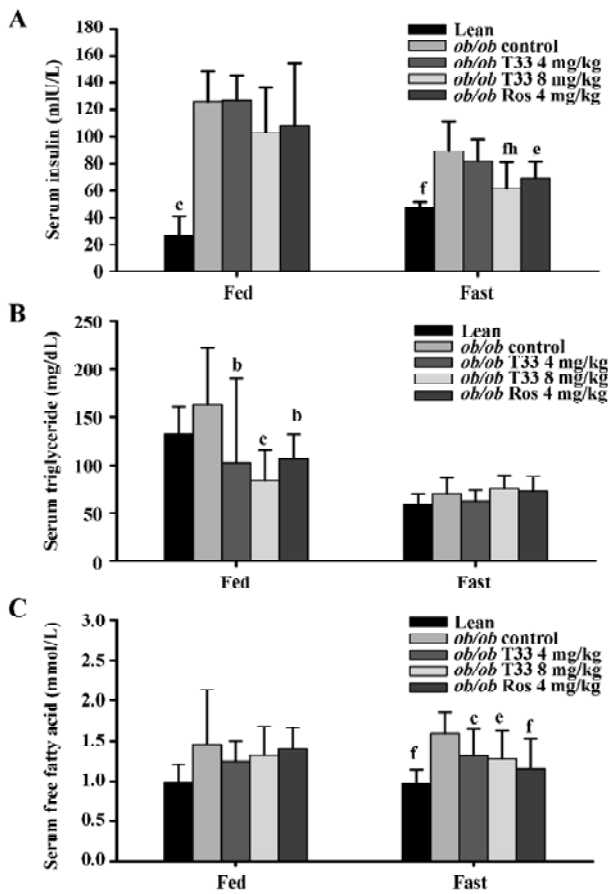
The *ob/ob* mice showed elevated FFA levels when compared with the lean mice (Figure 2C). After 20 d treatment with T33, the serum FFA levels in the *ob/ob* mice that were made to fast was significantly reduced as compared with that of the control mice ( $P<0.05$ ). However, no significant decrease was observed in the fed mice (Figure 2C). Rosiglitazone treatment led to a significant ( $P<0.05$ ) reduction in serum FFA concentration in the fasted mice, but not in the fed mice.

During the whole treatment, an increase in body weight was observed in the *ob/ob* mice. No significant difference could be found between the control and T33 or rosiglitazone-treated groups, which suggests that there is no T33 treatment-related effect on the body weight of *ob/ob* mice (Table 4).

**Effect of T33 on the triglyceride deposition in the skeletal muscle and liver** To investigate the lipid deposition in

**Table 3.** Effects of T33 on blood glucose values (mmol/L) of ITT in *ob/ob* mice.  $n=8-12$ . Mean±SD. <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs control group of *ob/ob* mice. <sup>e</sup> $P<0.05$ , <sup>f</sup> $P<0.01$  vs 4 mg/kg T33 group of *ob/ob* mice. <sup>h</sup> $P<0.05$ , <sup>i</sup> $P<0.01$  vs 0 min of each group.

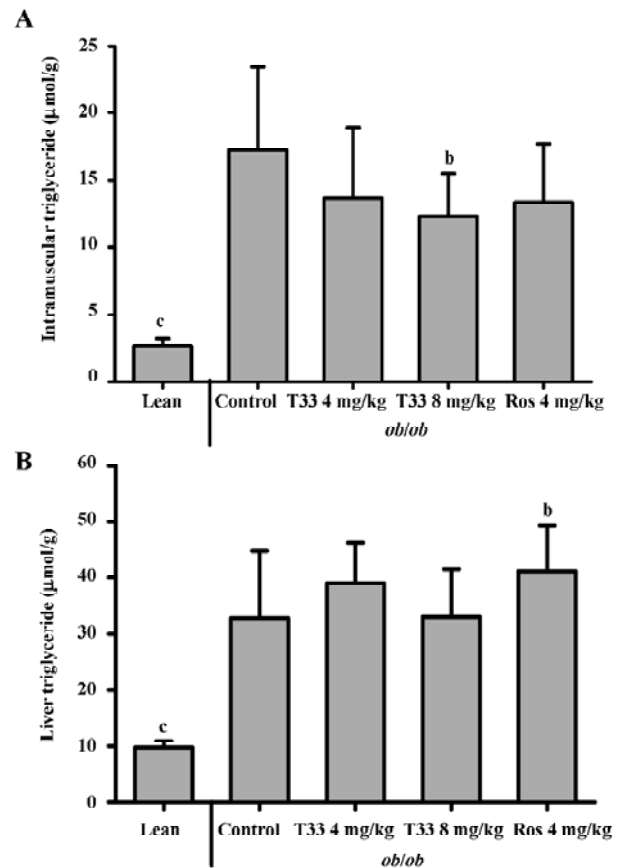
Group	Dose (mg/kg)		Time after insulin injection (min)					
			0	15	40	90	120	240
Control	-	Blood glucose value (mmol/L)	12.91±5.43	13.85±6.57	11.25±7.06	10.73±7.01	12.22±7.52	11.69±6.53
		Percentage (%) vs 0 min	100.00±0.00	105.98±17.85	81.71±30.20	76.78±31.56 <sup>b</sup>	88.45±32.98	81.92±19.68
T33	4	Blood glucose value (mmol/L)	7.41±1.76 <sup>b</sup>	9.66±1.56 <sup>h</sup>	5.77±2.33 <sup>b</sup>	6.40±1.11	6.77±0.69 <sup>b</sup>	7.26±2.72
		Percentage (%) vs 0 min	100.00±0.00	136.10±38.42 <sup>h</sup>	81.90±42.19	89.88±24.93	95.67±22.56	101.51±41.29
T33	8	Blood glucose value (mmol/L)	5.25±1.44 <sup>c</sup>	3.90±2.20 <sup>c</sup>	2.95±1.34 <sup>ch</sup>	3.10±2.34 <sup>ch</sup>	3.30±2.37 <sup>c</sup>	4.15±1.53 <sup>c</sup>
		Percentage (%) vs 0 min	100.00±0.00	71.13±23.88 <sup>bh</sup>	55.51±16.46 <sup>i</sup>	53.99±27.13 <sup>ei</sup>	58.23±27.21 <sup>bei</sup>	78.21±11.26
Ros	4	Blood glucose value (mmol/L)	5.17±0.74 <sup>c</sup>	3.92±2.06 <sup>c</sup>	2.75±0.95 <sup>ci</sup>	2.38±2.11 <sup>ci</sup>	2.63±1.68 <sup>ci</sup>	3.55±0.52 <sup>c</sup>
		Percentage (%) vs 0 min	100.00±0.00	75.18±36.17 <sup>bf</sup>	53.92±18.08 <sup>i</sup>	45.21±38.34 <sup>bei</sup>	50.57±30.03 <sup>bei</sup>	69.48±10.97 <sup>e</sup>



**Figure 2.** Effects of T33 on serum insulin, triglyceride and FFA levels in *ob/ob* mice. The *ob/ob* mice were treated with T33 (4 mg/kg, 8 mg/kg), rosiglitazone (4 mg/kg) or vehicle (1% CMC) by gavage once daily for 20 d, and serum insulin, triglyceride and FFA values were measured. (A) Serum insulin values; (B) Serum triglyceride values; (C) Serum FFA values.  $n=8-12$ . Mean $\pm$ SD. <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs control group of *ob/ob* in fed mice, <sup>e</sup> $P<0.05$ , <sup>f</sup> $P<0.01$  vs control group of *ob/ob* mice that were made to fast. <sup>h</sup> $P<0.05$  vs 4 mg/kg T33 group of *ob/ob* mice that were made to fast.

the skeletal muscle and liver after T33 and rosiglitazone treatment in the *ob/ob* mice, the triglyceride content in the skeletal muscle and liver was evaluated. The triglyceride con-

tent in the skeletal muscle and liver of the *ob/ob* mice was much higher than that of the lean mice. After 20 d treatment with T33 at a dose of 8 mg/kg, the triglyceride content in the skeletal muscle of the *ob/ob* mice was reduced significantly ( $P<0.05$ ; Figure 3A). In contrast with its effect on intramuscular lipid deposition, T33 did not have any effect on triglyc-



**Figure 3.** Effects of T33 on triglyceride content in the skeletal muscle and liver of *ob/ob* mice. The *ob/ob* mice were treated with T33 (4 mg/kg, 8 mg/kg), rosiglitazone (4 mg/kg) or vehicle (1% CMC) by gavage once daily for 20 d; triglyceride content in the skeletal muscle and liver were measured. (A) Intramuscular triglyceride content; (B) Liver triglyceride content.  $n=8-12$ . Mean $\pm$ SD. <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs control group of *ob/ob* mice.

**Table 4.** Effects of T33 on the body weight of *ob/ob* mice.  $n=8-12$ . Mean $\pm$ SD.

Group	Dose(mg/kg)	d 0	d 4	d 8	d 12	d 16
Control	-	43.1 $\pm$ 3.4	45.4 $\pm$ 3.2	47.3 $\pm$ 2.9	50.0 $\pm$ 3.7	50.7 $\pm$ 4.1
T33	4	43.4 $\pm$ 5.4	45.6 $\pm$ 5.0	47.1 $\pm$ 4.7	50.6 $\pm$ 4.3	50.6 $\pm$ 4.3
T33	8	43.2 $\pm$ 4.1	45.2 $\pm$ 4.1	47.6 $\pm$ 3.9	51.5 $\pm$ 3.4	51.6 $\pm$ 3.7
Ros	4	43.0 $\pm$ 4.0	44.7 $\pm$ 3.9	46.3 $\pm$ 3.9	49.5 $\pm$ 3.9	51.0 $\pm$ 3.7

eride content in the liver of the *ob/ob* mice. However, rosiglitazone at a dose of 4 mg/kg significantly increased the hepatocyte lipid deposition in the *ob/ob* mice after 20 d treatment (Figure 3B).

## Discussion

Insulin resistance in type 2 diabetes is associated with both hyperglycemia and hyperlipidemia. PPAR are ligand-activated transcription factors, which offer a promising therapeutic approach for the treatment of different diseases. Fibrates and TZD used in the treatment of dyslipidemia and diabetes are ligands for PPAR $\alpha$  and PPAR $\gamma$ , respectively. Therefore, a PPAR $\gamma/\alpha$  dual agonist should facilitate better management of insulin resistance, hyperglycemia and hyperlipidemia of type 2 diabetes. T33, a benzopyran derivative with a different chemical structure to TZD, could increase the triglycerides accumulation in 3T3-L1 adipocytes and shows dual activation of  $\gamma$  and  $\alpha$  isoforms of PPAR in a cell-based reporter gene assay<sup>[16]</sup>. In the present study, we evaluated the antidiabetic effects of T33 in *ob/ob* mice. As the most potent, efficacious, less toxic and marketed TZD derivative<sup>[10,17]</sup>, rosiglitazone was chosen as the positive control in the present study. Since the ED<sub>50</sub> value of the hypoglycemic effect of rosiglitazone in *ob/ob* mice had been reported to be 3.6 mg/kg<sup>[17]</sup>, the dose of rosiglitazone was set at 4 mg/kg. The same, and a much higher dose of T33, were used to compare its effect with rosiglitazone.

*Ob/ob* mice, a model of severe obesity, insulin resistance, and diabetes caused by leptin deficiency<sup>[18]</sup>, was the commonly used animal model for the evaluation of antidiabetic and insulin-sensitizing drugs. The *ob/ob* mice developed remarkable hyperinsulinemia and relatively mild hyperglycemia at the age of 6 weeks; the hyperglycemia could only be maintained until 14 weeks. In the present study, T33 showed dose-dependent reduction in blood glucose levels. T33 8 mg/kg reduced the blood glucose concentration to the normal level, which is similar to the hypoglycemic efficacy of rosiglitazone at a dose of 4 mg/kg. Among the PPAR $\gamma$  agonists in the market, rosiglitazone is claimed to be the most potent and efficacious. However, there were several PPAR $\alpha/\gamma$  dual agonists which showed more potent hypoglycemic effects than rosiglitazone, although they were less potent in the activation of PPAR $\gamma$ <sup>[17,19]</sup>. Therefore, we speculate that the activation of PPAR $\alpha$  of PPAR $\alpha/\gamma$  dual agonists play an important role in their hypoglycemic effect.

The severely impaired glucose tolerance and insulin tolerance confirmed the insulin resistant state of the *ob/ob* mice. T33 treatment showed marked amelioration in oral glucose tolerance and insulin tolerance in the *ob/ob* mice, which sug-

gests potent insulin-sensitizing properties of this compound. Compensatory hyperinsulinemia, another characteristic of type 2 diabetes, appears to contribute to the development of many other disorders such as dyslipidemia and hypertension. *Ob/ob* mice exhibit marked hyperinsulinemia, and the T33 treatment reduced the serum insulin concentration significantly in the fed mice and fasted mice, which also suggests the insulin-sensitizing effect of T33 on *ob/ob* mice. Therefore, all these results suggest that T33 could improve insulin resistance and increase the whole body insulin sensitivity.

The metabolic profile of type 2 diabetes included impaired glucose metabolism and insulin resistance, frequently combined with dyslipidemia<sup>[20]</sup>. In an insulin-resistant condition, lipolysis in the peripheral adipose tissue increased, enhancing FFA production and leading to a high plasma FFA level<sup>[2]</sup>. Many tissues, such as the liver and skeletal muscle, exposed on the high plasma FFA level, can cause and progress insulin resistance, since FFA can switch to TG accumulation in liver and skeletal muscles, which impacts insulin action<sup>[21]</sup>. In the present study, T33 exhibited potent lipid-lowering efficacy in the *ob/ob* mice. The lower FFA and TG levels in peripheral circulation may contribute to the insulin-sensitizing effect of T33.

Decreased plasma FFA oxidation and increased FFA flux from peripheral tissues to the liver contribute to hepatic steatosis, which is an important factor associated with insulin resistance<sup>[22]</sup>. Rosiglitazone had been found to produce moderate to severe fatty liver in rodents<sup>[23]</sup>. In the present study, the TG content in the liver of the *ob/ob* mice was markedly increased when compared with the lean mice. Treatment with rosiglitazone at a dose of 4 mg/kg could further increase hepatocyte lipid deposition, whereas T33 at the same dose as rosiglitazone produced a lower degree of hepatocyte lipid accumulation. Moreover, T33 at a dose of 8 mg/kg did not increase TG content in the liver, which suggests that T33 had an improved side effect profile compared to rosiglitazone.

The skeletal muscle and adipose tissues are 2 major peripheral tissues that account for whole body glucose utilization. Under insulin-stimulated conditions, approximately 80% of glucose disposal occurs in the skeletal muscle, whereas adipose tissues account for a smaller fraction of whole body glucose uptake<sup>[24,25]</sup>. Increasing evidences show that the accumulation of TG in skeletal muscles may contribute to the impaired insulin regulated metabolic actions, including glucose transport, glycogen synthesis and gene expression<sup>[26]</sup>. In the present study, the TG content in the skeletal muscle was significantly higher than that of the lean mice, which is consistent with the impaired glucose uptake

in the soleus muscle of those mice (unpublished data). T33 treatment at a dose of 4 and 8 mg/kg for 20 d could significantly decrease the intramuscular TG in *ob/ob* mice, with rosiglitazone exhibiting similar effects. Our unpublished data show that the treatment of *ob/ob* mice for 20 d with T33 at a dose of 8 mg/kg could increase both basal and insulin-stimulated glucose uptake in the soleus muscle and increased insulin signaling in the EDL muscle. Therefore, T33 could reduce lipid deposit in the skeletal muscle, which might contribute to the insulin-sensitizing effect of this compound on the skeletal muscle.

In summary, the PPAR $\gamma$ / $\alpha$  dual agonist T33 exerts potent and efficacious hypoglycemic, hypolipidemic and insulin-sensitizing effects in *ob/ob* mice. It not only lowered lipids in peripheral circulation, but also reduced triglyceride deposit in the skeletal muscle. We believe that T33 is a promising antidiabetic compound that will be helpful for the treatment of type 2 diabetes. Further studies should be carried out to develop T33 as a novel therapy for type 2 diabetes.

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