

## Hypoglycemic Agent YM440 Ameliorates the Impaired Hepatic Glycogenesis After Glucose Loading by Increasing Glycogen Synthase Activity in Obese Zucker Rats

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**ABSTRACT**—We studied the role of hepatic glycogenesis in glucose intolerance after glucose loading in obese Zucker rats and the effects of YM440 ((Z)-1,4-bis{4-[(3,5-dioxo-1,2,4-oxadiazolidin-2-yl)methyl]phenoxy}but-2-ene) on it. Lean and obese Zucker rats were treated with YM440 (300 mg/kg) for 14 days and then fasted for 20 h. Thirty percent glucose (0.6 g/kg) or saline was administered intravenously followed by NaH<sup>14</sup>CO<sub>3</sub>. Gluconeogenesis was evaluated based on the incorporation of <sup>14</sup>C-bicarbonate into blood glucose and hepatic glycogen. Obese rats showed an increase in the incorporation of <sup>14</sup>C into blood glucose of 2.5-fold compared to lean rats. The glucose loading decreased the <sup>14</sup>C-blood glucose release by 18% in obese rats and 43% in lean rats at 45 min. Glucose loading increased the hepatic glycogen content and <sup>14</sup>C incorporation into glycogen in lean but not obese rats. YM440 decreased levels of fasting plasma insulin and blood glucose and the hepatic glycogen content by 50% compared with values for untreated obese rats. After glucose loading, YM440 promoted the incorporation of <sup>14</sup>C into glycogen and glycogen synthase activity, leading to an improvement in glucose tolerance. These results indicate that glucose intolerance in obese rats was associated with decreased hepatic glycogenesis and YM440 improved the intolerance by normalizing glycogen metabolism.

**Keywords:** Gluconeogenesis, Glycogen, Glucose intolerance, Glycogen metabolism, Liver

Insulin resistance is one of the most important factors in the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM) (1, 2). Obese Zucker rats are reported to be an animal model of insulin resistance characterized by hyperinsulinemia and glucose intolerance (3, 4). Insulin plays a pivotal role in regulating glucose turnover in vivo. It stimulates glucose uptake in the peripheral tissues and also glycogen synthesis in the liver (5) and muscle (6). Insulin decreases gluconeogenesis in the liver (7). Although the important role of insulin in regulating glucose and glycogen metabolism is recognized, the precise mechanism of insulin resistance still remains unknown. Since insulin resistance may be due to a combination of defects in the action of insulin, it is necessary to develop methods that detect such abnormalities. Glucose tolerance is also known to be regulated by several factors related to the action of insulin and mass action of glucose such as insulin secretion, peripheral

glucose uptake, hepatic glucose production and hepatic gluconeogenesis (8). Therefore the glucose tolerance test is one of the most suitable models with which to examine causes of insulin resistance in animals. Several studies showed a significant role for hepatic glucose production in glucose tolerance: glucose loading changed the final product of hepatic gluconeogenesis from blood glucose to liver glycogen in vivo (8, 9). Shikama and Ui (8) demonstrated that the intravenous or oral administration of a glucose solution into fasted rats simultaneously injected with <sup>14</sup>C-bicarbonate resulted in an inhibition in the release of <sup>14</sup>C-glucose into the blood and an inhibition in the formation of <sup>14</sup>C-glycogen associated with activation of glycogen synthase in the liver. Since the specific activity of <sup>14</sup>C-glycogen was much higher than that of <sup>14</sup>C-blood glucose after glucose loading, glycogen was synthesized mainly from the product of gluconeogenesis rather than blood glucose. The combination of tracer kinetics using <sup>14</sup>C-bicarbonate and glucose loading is suitable for detecting the role of gluconeogenesis in glucose intolerance in animals

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with insulin resistance.

Our previous study (10) showed that (Z)-1,4-bis{4-[(3,5-dioxo-1,2,4-oxadiazolidin-2-yl)methyl]phenoxy}but-2-ene (YM440), an insulin sensitizer with oxadiazolidinediones, decreased hyperglycemia in diabetic db/db mice without changing body weight. YM440 had no effect on adipocyte differentiation and was not a ligand for PPAR $\gamma$  (peroxisome proliferator-activated receptor  $\gamma$ ). The aim in the present study was to examine the role of hepatic glucose metabolism in regulating glucose tolerance in obese Zucker rats. In combination with tracer kinetics using NaH<sup>14</sup>CO<sub>3</sub> and oral glucose tolerance, we measured the gluconeogenic activity, the amount of gluconeogenic product incorporated into blood glucose and hepatic glycogen, and the activities of glycogen metabolizing enzymes. We also examined the mechanism by which YM440 ameliorates glucose intolerance in these obese animals.

## MATERIALS AND METHODS

### Materials

YM440 was synthesized at Yamanouchi Pharmaceutical Co. (Tokyo). NaH<sup>14</sup>CO<sub>3</sub> and UDP-[<sup>3</sup>H]-glucose were purchased from Dupon/NEN (Boston, MA, USA) and Amer-sham Japan (Tokyo), respectively. Other reagents were of analytical grade from commercial sources.

### Animals and treatments

Male Zucker fatty (fa/fa) rats and their lean littermates (Fa/–) (5 weeks of age) were obtained from Charles River Laboratories (Kingston, NY, USA) and allowed free access to laboratory chow (CE-2, 341 kcal/100 g; CLEA Japan, Tokyo). The animals were maintained in a 12:12 h light-dark cycle. The experimental protocol was approved by the local ethical committee for animal studies. The rats aged 15 weeks were orally administered YM440, suspended in 0.5% methylcellulose solution (5 ml/kg), once a day at a dose of 300 mg/kg for 14 days. The vehicle was administered to control rats. In separate studies using obese Zucker rats, YM440 significantly improved glucose tolerance at a dose of 300 mg/kg, but not at a dose of 100 mg/kg, although this agent decreased hyperglycemia in a dose-dependent manner (30, 100, 300 mg/kg). Before treatment, there was no significant difference between the vehicle- and YM440-treated groups in blood glucose, plasma insulin and body weight. The rats were fasted for 20 h after the last dosing.

### Analytical methods

Before glucose loading, blood specimens were obtained from the tail vein and blood glucose and plasma insulin levels were determined. Gluconeogenic activity was measured as described previously (8). At time 0, a solution of

30% glucose (0.6 g/2 ml per kg) was administered intravenously into the femoral vein under pentobarbital anesthesia (45 mg/kg, i.p.) and followed by NaH<sup>14</sup>CO<sub>3</sub> (3  $\mu$ mol and 20  $\mu$ Ci/100 g in saline) after 5 min. Blood specimens (0.1 ml) were taken from the tail vein at intervals. The collected blood was hemolyzed in 1.5 ml of distilled water and then deproteinized by Ba(OH)<sub>2</sub> and ZnSO<sub>4</sub> before centrifugation at 2,000  $\times$  g for 15 min. Glucose concentrations in the supernatant were measured by the glucose oxidase method (Glucose mono test; Roche, Tokyo). The radioactivity of <sup>14</sup>C-glucose in the supernatant, which was not absorbed by Dowex 50-X8 (H<sup>+</sup> form) and AG1-X8 (formate form) (11), was determined using a liquid scintillation counter.

For the liver biopsy, the abdominal cavity was opened under pentobarbital anesthesia, and a portion of the liver was rapidly removed and immediately frozen in liquid nitrogen 50 min after glucose loading. The frozen tissue was analyzed for glycogen (12) and <sup>14</sup>C-glycogen (11). Briefly, pieces of frozen tissue (about 300 mg) were placed in 1 ml of a 30% KOH solution and dissolved in a boiling water-bath. Then, 2 ml of ethanol was added to the tube and mixed well. The tube was kept overnight at 4°C and then centrifuged at 2,000  $\times$  g for 15 min. The supernatant was decanted and the resulting pellet was dissolved in 1 ml of water. An aliquot of this solution, 5 or 50  $\mu$ l, was diluted to 1 ml with water and then 2 ml of anthrone reagent (0.2 g anthrone/100 ml 95% H<sub>2</sub>SO<sub>4</sub>) was added. After mixing vigorously, the tubes were boiled for 10 min and the optical density was determined at 620 nm. Glycogen (10 mg/tube) was added to the remaining glycogen solution as a carrier and precipitated twice by the addition of ethanol and centrifugation. The radioactivity of <sup>14</sup>C-glycogen was measured using a liquid scintillation counter.

The activity of phosphorylase a was determined in the presence of caffeine (13). Frozen liver was homogenized in a 100 mM Tris-HCl buffer (pH 7.4) containing 20 mM NaF, 20 mM EDTA and 0.5% glycogen. The resultant 10% homogenate (0.05 ml) was mixed with 0.05 ml of incubation medium, which was prepared by freshly dissolving 0.1 M glucose 1-phosphate, 2% glycogen, 1 mM caffeine and 7.5 mM cysteine-HCl in 35 mM glycerol 2-phosphate solution containing 1 mM EDTA and 20 mM NaF. The mixture was incubated at 20°C for 15 min. The reaction was stopped by the addition of 30% HClO<sub>4</sub>, and the acidified mixture was diluted with 2 ml of water before centrifugation. The protein-free supernatant was measured for P<sub>i</sub> (14). One unit of phosphorylase is the amount of enzyme that liberates 1  $\mu$ mol of P<sub>i</sub> per min under the conditions of the assay. Glycogen synthase activity was determined as described previously (15). Briefly, 0.05 ml of 5% liver homogenate in a 50 mM Tris-HCl buffer (pH 7.8) containing 0.4  $\mu$ mol and 0.1  $\mu$ Ci [<sup>3</sup>H]-UDP-glucose and 1 mg of

glycogen in a total volume of 0.1 ml was incubated for 10 min at 20°C in the presence (total activity of glycogen synthase) or absence (active form of glycogen synthase) of 0.8  $\mu$ mol of glucose 6-phosphate. Then an aliquot of the reaction mixture was immediately spotted on filter paper and washed in ice-cold 66% ethanol. After 40 min of mild stirring, ethanol was discarded and the papers were washed twice with ethanol and dried by acetone.

#### Data analyses

Data were analyzed by one-way or two-way analysis of variance. When differences were statistically significant ( $P < 0.05$ ), Tukey's multiple range test was used to compare values between experimental groups. Differences were accepted as significant at the  $P < 0.05$  level.

## RESULTS

#### Effects of YM440 on body and liver weights and on plasma concentrations of insulin and blood glucose

Body and liver weights and plasma concentrations of insulin were higher in obese than lean Zucker rats (Table 1). YM440 treatment for 14 days had no effect on body and liver weights in lean or obese rats, but significantly reduced plasma insulin levels in obese rats. As shown in Fig. 1B, in obese rats, YM440 significantly decreased the blood glucose level at time 0 compared with obese rats. Such a change, however, was not observed 10 min after an injection of vehicle. YM440 reduced hyperglycemia after glucose loading in obese rats, but only slightly increased

**Table 1.** Effects of YM440 on body weight, liver weight and plasma insulin levels in Zucker lean and obese rats

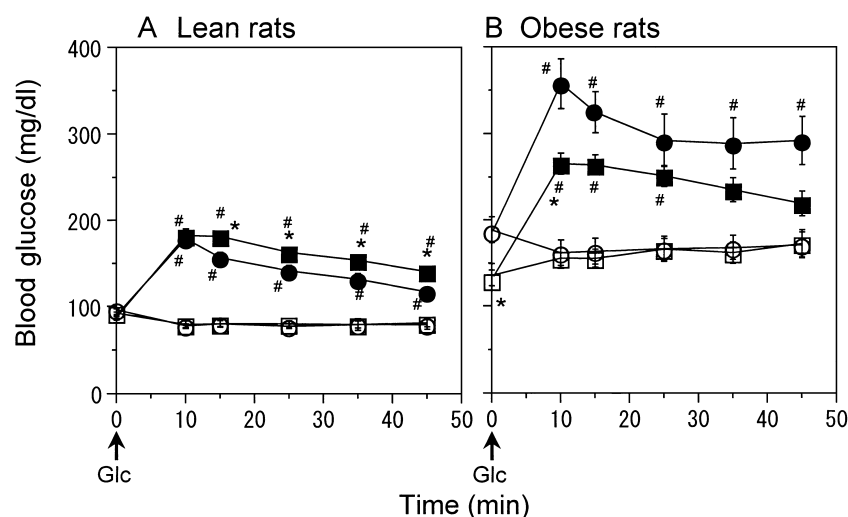
	No. of animals	Body weight (g)	Liver weight (g)	Plasma insulin (ng/ml)
Lean rats				
Vehicle	16	411 $\pm$ 7	10.8 $\pm$ 0.4	5.18 $\pm$ 0.44
YM440	17	397 $\pm$ 8	10.7 $\pm$ 0.6	2.78 $\pm$ 0.29
Obese rats				
Vehicle	18	594 $\pm$ 14 <sup>#</sup>	35.3 $\pm$ 1.6 <sup>#</sup>	23.6 $\pm$ 3.10 <sup>#</sup>
YM440	17	587 $\pm$ 17	30.9 $\pm$ 1.8	6.81 $\pm$ 0.65*

Values are the mean  $\pm$  S.E.M. Rats were treated with YM440 (300 mg/kg) or a vehicle for 14 days and then fasted for 20 h after the last dosing. <sup>#</sup> $P < 0.05$  vs lean rats, \* $P < 0.05$  vs vehicle control. Before treatment, there was no significant difference in plasma insulin and body weight between the vehicle- and YM440-treated groups in lean or obese rats.

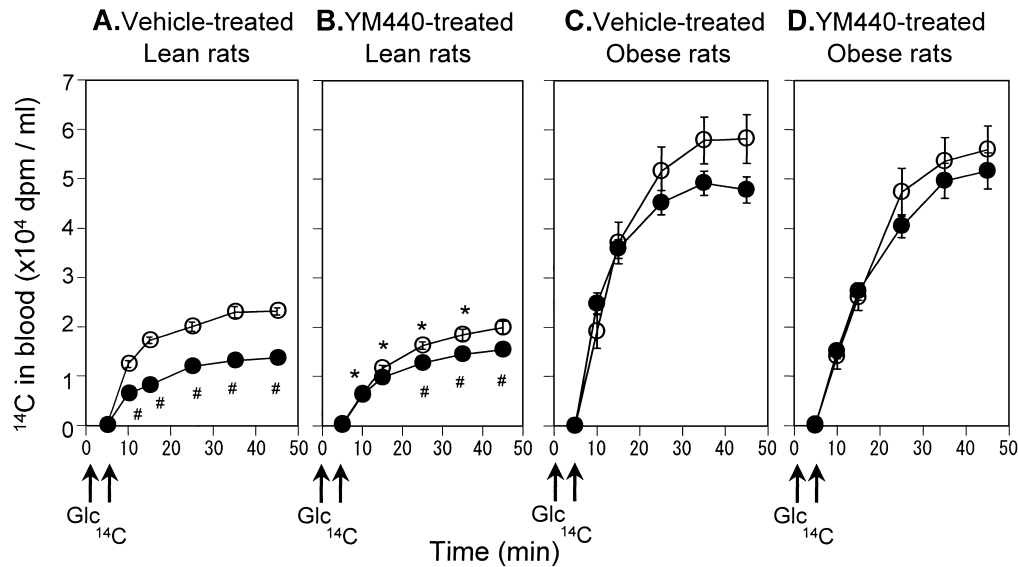
blood glucose levels in lean rats (Fig. 1: A and B).

#### Effects of YM440 on incorporation of $^{14}$ C from $^{14}$ C-bicarbonate into blood glucose and hepatic glycogen and on glycogen metabolizing enzymic activities

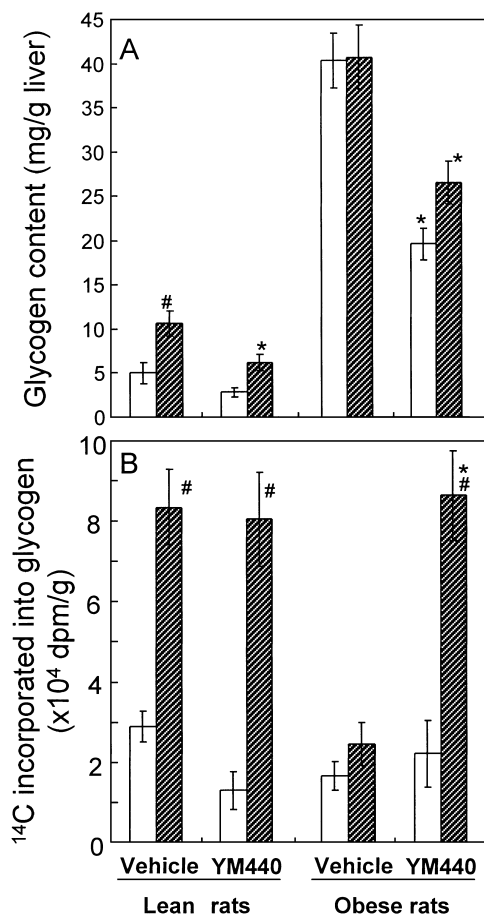
As shown in Fig. 2, A and C, obese rats showed an increase in the incorporation of  $^{14}$ C from  $^{14}$ C-bicarbonate into blood glucose by about 2.5-fold compared to lean rats. Glucose loading decreased  $^{14}$ C-glucose release in lean and obese rats by 45% and 18%, respectively, at 45 min, but the latter reduction was not significant. In lean rats without glucose loading (Fig. 2: A and B), YM440 de-



**Fig. 1.** Effects of YM440 on blood glucose levels with or without intravenous glucose loading in fasted Zucker lean and obese rats. Rats were treated with YM440 (300 mg/kg) or a vehicle for 14 days and then fasted for 20 h after the last dosing. A solution of 30% glucose or saline (0.6 g/2 ml per kg) was administered intravenously at time 0 and blood samples were taken from the tail vein at the indicated times. Vehicle-treated rats with (closed circle) or without (open circle) glucose loading and YM440-treated rats with (closed square) or without (open square) glucose loading are shown. Values are the means  $\pm$  S.E.M. (n = 8 or 9). Effect of glucose loading: <sup>#</sup> $P < 0.05$  vs saline, effect of YM440: \* $P < 0.05$  vs vehicle control. Glc: glucose.



**Fig. 2.** Effects of YM440 on the incorporation of  $^{14}\text{C}$  from bicarbonate into blood glucose with or without intravenous glucose loading in fasted Zucker lean and obese rats. Lean rats (A, B) and obese rats (C, D) were treated with either vehicle or YM440 (300 mg/kg) for 14 days and fasted for 20 h after the last dosing. A solution of 30% glucose or saline (0.6 g/2 ml per kg), and  $^{14}\text{C}$ -bicarbonate were administered intravenously at time 0 and 5 min, respectively. Blood samples were taken from the tail vein at the indicated times and the radioactivity of  $^{14}\text{C}$ -glucose was measured in a liquid scintillation counter. Rats with (closed circle) or without (open circle) glucose loading are shown. Values are the means  $\pm$  S.E.M. ( $n = 8$  or  $9$ ). Effect of glucose loading:  $^{\#}P < 0.05$  vs saline, effect of YM440:  $*P < 0.05$  vs vehicle control. Glc: glucose,  $^{14}\text{C}$ :  $^{14}\text{C}$ -bicarbonate.



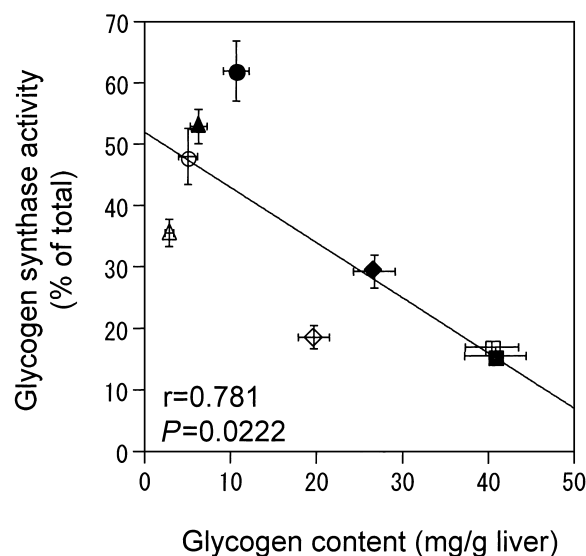
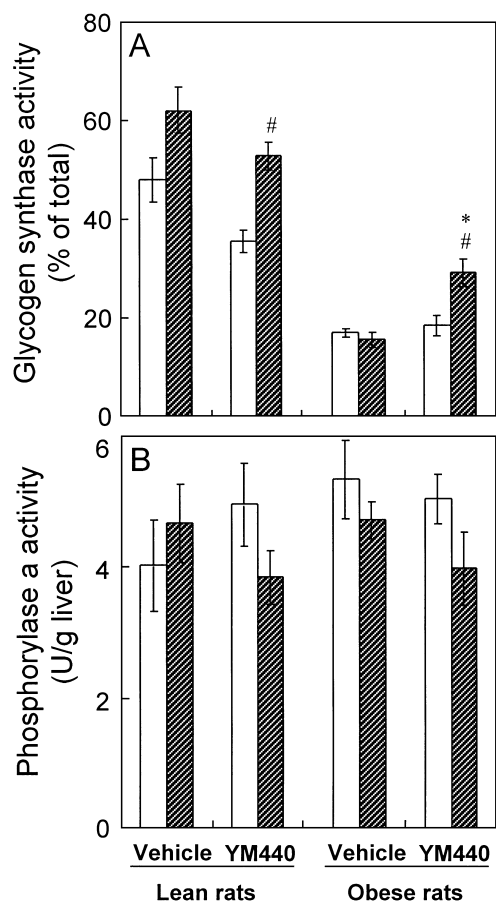
creased  $^{14}\text{C}$ -glucose release compared with the normal control and after glucose loading, the release was significantly reduced compared with the respective control rats treated with or without YM440. In contrast, in obese rats, YM440 had no significant effect on  $^{14}\text{C}$ -glucose release with or without glucose loading (Fig. 2D). In Fig. 3, glucose loading increased hepatic glycogen content and incorporation of  $^{14}\text{C}$  into glycogen in lean rats treated with and without YM440. Although neither hepatic glycogen content nor incorporation of  $^{14}\text{C}$  into glycogen was increased in obese rats after glucose loading, YM440 significantly restored the incorporation of  $^{14}\text{C}$  into glycogen in obese rats after glucose loading. The recovery of  $^{14}\text{C}$ -incorporation into glycogen was associated with an approximate 50% reduction in hepatic glycogen content compared with that in obese rats treated with vehicle. As shown in Table 2, the specific activities of the  $^{14}\text{C}$ -glucose of blood glucose and

**Fig. 3.** Effects of YM440 on hepatic glycogen content (A) and the incorporation of  $^{14}\text{C}$  from bicarbonate into hepatic glycogen (B) with or without intravenous glucose loading in fasted Zucker lean and obese rats. The experimental conditions are shown in Fig. 2. At 50 min after glucose loading, the liver was excised and the hepatic glycogen content and incorporation of  $^{14}\text{C}$  from  $^{14}\text{C}$ -bicarbonate into hepatic glycogen in Zucker lean and obese rats with (hatched bar) or without (open bar) glucose loading were determined. Values are the means  $\pm$  S.E.M. ( $n = 8$  or  $9$ ). Effect of glucose loading:  $^{\#}P < 0.05$  vs saline, effect of YM440:  $*P < 0.05$  vs vehicle control.

**Table 2.** Specific activity of  $^{14}\text{C}$ -glucose of blood glucose and hepatic glycogen

Treatment		Blood glucose (dpm/mg)	Liver glycogen (dpm/mg)
Lean rats			
Vehicle	saline	29,901 $\pm$ 1,469	9,476 $\pm$ 3,272
	glucose	11,753 $\pm$ 377 <sup>#</sup>	8,510 $\pm$ 1,044
YM440	saline	24,600 $\pm$ 851*	6,330 $\pm$ 3,275
	glucose	10,922 $\pm$ 426 <sup>#</sup>	14,336 $\pm$ 2,540
Obese rats			
Vehicle	saline	35,342 $\pm$ 2,957	502 $\pm$ 181
	glucose	17,226 $\pm$ 1,452 <sup>#</sup>	660 $\pm$ 159
YM440	saline	34,049 $\pm$ 3,427	1,216 $\pm$ 519
	glucose	23,507 $\pm$ 649 <sup>#</sup>	3,425 $\pm$ 544 <sup>#</sup> *

Values are the mean  $\pm$  S.E.M. (n = 8 or 9). Rats were treated with YM440 (300 mg/kg) or vehicle for 14 days and then fasted for 20 h after the last dosing. A solution of 30% glucose or saline (0.6 g / 2 ml per kg) and  $^{14}\text{C}$ -bicarbonate were administered intravenously at 0 and 5 min, respectively. Blood and liver samples were taken at 45 and 50 min, respectively. Effect of glucose loading: <sup>#</sup> $P < 0.05$  vs saline, effect of YM440: \* $P < 0.05$  vs vehicle control.



**Fig. 5.** Correlation of glycogen synthase activity with hepatic glycogen content. Points represent the mean  $\pm$  S.E.M. obtained in Figs. 3 and 4. A line was drawn by the least-squares fitting method. Zucker lean rats with YM440 (triangle) or vehicle (circle) and obese rats with YM440 (diamond) or vehicle (square) with (closed symbols) or without (open symbols) glucose loading.

hepatic glycogen might be changed by several factors, such as YM440 treatment, obesity, and glucose loading. The specific activity of hepatic glycogen in each group was not significantly higher than the corresponding value of blood glucose, suggesting that some of the glucose molecules in glycogen were not directly derived from gluconeogenic precursors, but rather blood glucose. Glucose loading did not change the activities of glycogen phosphorylase in lean and obese rats (Fig. 4B). However, in lean rats and obese rats treated with YM440, glucose loading significantly increased glycogen synthase activity, but this enzymic activity in obese rats treated with vehicle was low and was not activated after glucose loading (Fig. 4A).

#### Significant correlation between glycogen content and glycogen synthase activity

As shown in Fig. 5, there was a significant correlation between glycogen synthase activity and hepatic glycogen content after glucose loading.

**Fig. 4.** Effects of YM440 on the activities of hepatic glycogen synthase (A) and glycogen phosphorylase (B) with or without intravenous glucose loading in fasted Zucker lean and obese rats. The liver sample used in this study was the same as in Fig. 3. Hepatic glycogen synthase activity (% of total) and glycogen phosphorylase activity was assayed in Zucker lean and obese rats with (hatched bar) or without (open bar) glucose loading. Values are the means  $\pm$  S.E.M. (n = 8 or 9). Effect of glucose load: <sup>#</sup> $P < 0.05$  vs saline, effect of YM440: \* $P < 0.05$  vs vehicle control.

## DISCUSSION

In the present study, we examined gluconeogenic activity based on the incorporation of  $^{14}\text{C}$ -bicarbonate into blood glucose and the involvement of gluconeogenic products in glucose intolerance observed in obese Zucker rats. During the initial stage of gluconeogenesis in the liver, pyruvate is carboxylated with  $^{14}\text{CO}_2$  to yield oxaloacetate, which is in rapid equilibrium with malate, citrate, and aspartate (16). Labeled oxaloacetate is then converted to phosphoenolpyruvate in the presence of GTP. Labeled phosphoenolpyruvate is converted into glucose 6-phosphate in the pathway of gluconeogenesis. There are two pathways for the synthesis of glycogen in the liver (8, 9): directly from glucose, via glucose 6-phosphate, glucose 1-phosphate and UDP-glucose, and indirectly from the product of gluconeogenesis, via pyruvate, fructose 1,6-diphosphate and glucose 6-phosphate.

One striking finding of the present study was that hepatic glycogen synthesis after glucose loading was impaired in obese Zucker rats. Glucose loading increased glycogen synthesis rapidly in the liver and increased  $^{14}\text{C}$  incorporation from  $^{14}\text{C}$ -bicarbonate into liver glycogen concomitantly with a reduction in  $^{14}\text{C}$  incorporation into blood glucose in lean Zucker rats (Figs. 2 and 3). These findings are compatible with our previous observations that oral glucose loading resulted in an inhibition of  $^{14}\text{C}$ -glucose release into the blood and in an accelerated  $^{14}\text{C}$ -glycogen formation associated with glycogen synthase activation in the liver of Wistar rats (8). However, in obese Zucker rats, glucose loading had no effect on hepatic glycogen content and  $^{14}\text{C}$  incorporation into liver glycogen or blood glucose, although  $^{14}\text{C}$  incorporation into blood glucose was increased by about 2.5-fold in these obese rats compared with lean rats. Our previous study (8) indicated that increases in liver glycogen after glucose loading were mainly derived from gluconeogenesis rather than blood glucose because the specific activity of  $^{14}\text{C}$ -glycogen was higher than that of  $^{14}\text{C}$ -blood glucose. This study also indicated that a diversion of gluconeogenic products from blood to liver glycogen was an important determinant of glucose tolerance. In the present study, however, we could not confirm the origin of  $^{14}\text{C}$ -glycogen in the liver based on the specific activities of blood glucose and glycogen. There is no clear evidence that an accelerated  $^{14}\text{C}$ -glycogen formation in lean rats is due to a diversion of gluconeogenic products from blood to liver glycogen. The reason for this difference is unclear but the glycogen content in fasted lean Zucker rats was higher than that in Wistar rats (5.1 vs 2.4 mg/g). Since the accumulated glycogen may decrease the specific activity of glycogen, it is difficult to compare the specific activities of glucose and glycogen directly.

Clearly, it is difficult to explain the apparently conflict-

ing findings that an increased amount of glycogen and suppression of glycogen synthesis were observed in the liver of obese Zucker rats. It has been suggested that increased lipogenesis suppresses glycogenesis in obese Zucker rats (17). Noshiro et al. (3) showed that plasma levels of triglycerides and non-esterified fatty acid were increased in obese Zucker rats and such increases might be due to hyperinsulinemia. In addition, Obeid et al. (17) demonstrated that ingested carbohydrate was used predominantly for hepatic fatty acid synthesis in obese Zucker rats and that hepatic glycogen synthesis was suppressed. The suppression of hepatic glycogen synthesis may contribute to the increased energetic efficiency of obese animals. However the effect of YM440 on hepatic fatty acid synthesis and the relationship between glycogen synthesis and fatty acid synthesis remains to be determined. Although glycogen synthesis is suppressed, the 80% greater food intake in obese animals than in lean rats may cause a small but significant increase in glycogenesis in the liver (data not shown).

Koubi et al. (18) reported that liver glycogen was resistant to exhaustion in obese Zucker rats. After 48-h fasting or cold exposure (at 4–7°C), obese Zucker rats cannot mobilize hepatic glycogen stores, as observed in lean control rats. After 24 h of refeeding, hepatic glycogen stores were restored in lean rats, but decreased in obese rats compared with prefasting levels. It is likely that the high content of hepatic glycogen affects the metabolism of glycogen by regulating glycogen synthase activity (19). There is an inverse correlation between glycogen content and active glycogen synthase activity in rats with liver glycogen-storage disorders. Glycogen synthase phosphatase (GSP) activity is inhibited by glycogen. As shown in Fig. 3, glycogen synthase activity in the present study was low and not activated after glucose loading. Therefore, there is a possibility that the activity of GSP is inhibited in obese rats. However, it has been reported that hepatic GSP activity was increased in fasted obese Zucker rats at 10 weeks of age (20), which were younger than those used in the present study (17 weeks of age). The 20-h fasting decreased only 10% of glycogen content in fed obese rats ( $46.2 \pm 1.3$  mg/g liver) at 17 weeks of age (data not shown), but Van de Werve and Jeanrenaud (21) reported that about 80% of hepatic glycogen was decreased after 17-h fasting in obese rats at 10 weeks of age. These differences in hepatic glycogen content after fasting in obese rats suggest that hepatic glycogen metabolism is impaired with age. Therefore it is likely that the impairment in glycogen metabolism in obese rats used in this study was more severe than that in obese rats at 10 weeks of age reported by Van de Werve and Jeanrenaud. Further study will be needed to clarify the role of glycogen synthase phosphatase in glycogen metabolism in these obese rats.

Another interesting aspect of this study was that YM440 significantly increased the incorporation of  $^{14}\text{C}$  into glycogen in obese rats, who showed no effect on incorporation of  $^{14}\text{C}$  into glycogen after glucose loading when untreated. Its recovery was associated with an approximate 50% reduction in liver glycogen content and increased activity of glycogen synthase. However, YM440 slightly impaired glucose tolerance in lean rats compared with a vehicle-treated control. There is as yet no explanation for this effect of YM440 in lean rats. In addition, YM440 significantly reduced the plasma insulin levels in Zucker obese rats and improved glucose tolerance in Zucker obese rats. A part of these findings is compatible with our previous observations (10) using YM440, suggesting that this agent ameliorates insulin resistance in diabetic db/db mice. MCC-555, a novel thiazolidinedione ligand, is reported to reduce hyperglycemia and hyperinsulinemia in the diabetic KK-A $^y$  mouse model and administration of this agent to obese Zucker rats increased liver glycogen levels and glycogen synthase activity in the fed state (22). This agent had a tendency to enhance insulin-induced suppression of hepatic glucose production (HGP) during euglycemic hyperinsulinemic clamping, indicating that it improved metabolic abnormalities in glucose release from the liver.

Our previous findings indicated that, during euglycemic hyperinsulinemic clamping, YM440 as well as troglitazone partially decreased HGP under conditions where HGP in lean rats was suppressed to almost zero and HGP in untreated obese rats remained unchanged (23). It is suggested that the partial decrease in HGP in our previous study and the recovery of hepatic glycogenesis in the present study are due to the same mechanism of action of YM440. Although there is no explanation for the mechanism behind the abnormalities in glycogen metabolism in obese Zucker rats and a link between the changes in the reduced amount of liver glycogen and the increased activity of glycogen synthesis induced by YM440, the measurement of  $^{14}\text{C}$  from  $^{14}\text{C}$ -bicarbonate in blood glucose and hepatic glycogen can identify the step where the abnormality in glycogen metabolism exists. This technique also showed clearly that YM440 treatment in obese rats improved the deficit of glycogen metabolism.

In the present study, we examined the role of diversion of hepatic gluconeogenic products from blood glucose to liver glycogen in glucose intolerance in obese Zucker rats.  $^{14}\text{C}$  Incorporation into blood glucose was suppressed and  $^{14}\text{C}$  incorporation into hepatic glycogen was increased after glucose loading in lean controls. In contrast,  $^{14}\text{C}$  incorporation into blood glucose was increased in obese rats but not suppressed after glucose loading. YM440 had no significant effect on  $^{14}\text{C}$  incorporation into blood glucose but increased  $^{14}\text{C}$  incorporation into hepatic glycogen in obese rats. These changes were accompanied by an in-

crease in glycogen synthase activity and a reduction in hepatic glycogen content. These findings may indicate that YM440 ameliorates glucose intolerance by normalizing glycogen metabolism in obese rats with insulin resistance. Further study will be needed to clarify the precise mechanism by which YM440 improves glycogen metabolism directly or by secondary improvement of glucose and lipid metabolism.

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