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Slight but Significant Improvement of Insulin Resistance of Wistar Fatty Rats by Treatment with a Disaccharidase Inhibitor, AO-128

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TOMINAGA, M., KIMURA, M., IGARASHI, M., EGUCHI, H., IGARASHI, K., ABE, T., SUGIYAMA, K., MANAKA, H. and SASAKI, H. *Slight but Significant Improvement of Insulin Resistance of Wistar Fatty Rats by Treatment with a Disaccharidase Inhibitor, AO-128.* Tohoku J. Exp. Med., 1997, **181** (3), 353-360 —— To know whether the insulin resistance is improved by delaying carbohydrate absorption from the small intestine, we studied the effect of a disaccharidase inhibitor, AO-128, on insulin resistance of Wistar fatty rats. Rats were kept on standard laboratory chow with and without 10 ppm of AO-128 for 4 weeks, and then subjected to the glucose clamp. At the end of the 4-week treatment, plasma glucose level at 14 : 00 to 16 : 00 of AO-128 treated rats was 121 ± 14 mg/100 ml (mean $\pm$ S.D.), significantly lower than 226 ± 72 mg/100 ml of the rats without AO-128. During clamp steady state under 20 mU$\cdot$kg$^{-1}\cdot$min^{-1} continuous insulin infusion, glucose uptake of AO-128 treated rats was only 7.62 ± 0.70 mg$\cdot$kg$^{-1}\cdot$min^{-1}, not different from 6.64 ± 0.91 mg$\cdot$kg$^{-1}\cdot$min^{-1} of rats without AO-128, but much lower than the lean littermates (20.81 ± 3.11 mg$\cdot$kg$^{-1}\cdot$min^{-1}). However, the percent suppression of hepatic glucose output was $55.2 \pm 23.8\%$, which, though incomplete, was significantly higher than $17.4 \pm 11.2\%$ of rats without AO-128. The present study suggested that there were at least two, components of insulin resistance, a genetically determined and a poor-glycemic control-related, and that the latter insulin resistance was ameliorated by AO-128. ———— disaccharidase inhibitor; AO-128; insulin resistance; Wistar fatty rats; glucose clamp

Since both postprandial hyperglycemia and hyperinsulinemia were improved by treatment with a disaccharidase inhibitor in patients with non-insulin dependent diabetes mellitus (NIDDM), the possibility that this drug might improve insulin resistance was suggested. Indeed, Friedman et al. (1991) have reported an increase in glucose transporter (GLUT)-4 protein of Zucker fatty rats by an

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acarbose treatment, and Shinozaki et al. (1996) have found an improvement of in vivo sensitivity to insulin in nondiabetic hyperinsulinemic patients by administration of a disaccharidase inhibitor, AO-128 (voglibose) (Odaka et al. 1992; Goto et al. 1995), using a steady state plasma glucose (SSPG) method. Therefore, a disaccharidase inhibitor could be recommended to prescribe to obese and insulin resistant patients even without metabolic derangement in glucose homeostasis, i.e. diabetes mellitus. However, the relationship between the improvement of glycemic control and the amelioration of insulin resistance has not been fully elucidated, because the improvement of glycemic control should result in at least a partial improvement of insulin resistance, by removing the influence of so called "glucose toxicity". Attempting to investigate this relationship in the most severe conditions, we chose a genetically obese and diabetic Wistar fatty rat with a severe insulin resistantce and a very high levels of plasma insulin (Sugiyama et al. 1989). With and without treatment by a disaccharidase inhibitor, AO-128, the glycemic control and in vivo insulin sensitivity of Wistar fatty rats were examined, using the most precise method, glucose clamp technique.

Materials and Methods

One group ($n=6$) of Wistar fatty rats, kindly provided by Dr. H. Ikeda (Takeda Chemical Industry, Osaka), were given standard laboratory chow, CRF-1 (Clea Co., Tokyo), mixed with 10 ppm of AO-128 (Takeda Chemical Industry), and another group ($n=6$) of Wistar fatty rats were given CRF-1 without AO-128. These rats were kept in individual metabolic cages in our laboratory animal ward with controlled lighting (on between 6 : 00 and 18 : 00) and temperature (22°C) for 4 weeks. Every week, their body weight, food intake, urinary volume, urinary glucose secretion, and blood glucose levels were measured between 14 : 00 and 16 : 00 hr. After fasting for 20 and 24 hr, they were subjected to the glucose clamp studies. Age-matched lean littermates ($n=6$) were also studied by the glucose clamp technique.

A slightly modified method of the glucose clamp study, originally introduced by Andres et al. (1966) and DeFronzo et al. (1979), has been described earlier in detail (Tominaga et al. 1992, 1995). In brief, under anesthesia with 0.35 g/kg chloral hydrate ip, the bilateral femoral veins, the right jugular vein, and the left carotid artery were surgically exposed and cannulated with Silastic tubings, for infusion of ^{3}H-3-glucose (New England Nuclear Company, Boston, MA, USA), and 20% glucose and insulin solution, for continuous blood sampling to measure glucose level by a GM-1320 Glucose Monitor (Kyoto Daiichi Scientific Equipment Co., Kyoto), and for blood sampling to measure specific ^{3}H radioactivity, respectively. Immediately after surgery, a continuous infusion of 0.05 μCi of ^{3}H-glucose was started. After blood sampling to determine the glucose disappearance (Gd) during basal states by the method of Steele (1959), 20 mU$\cdot$kg$^{-1}\cdot$min^{-1} of porcine insulin was infused in a prime mode. The blood glucose levels

decreased to 140 mg/100 ml, then the infusion of 20% glucose solution began at a variable infusion rate to maintain the blood glucose levels at this target level for 60 min. At the end of clamp, the blood was sampled to determine both the specific radioactivity of ^3H and insulin level. Insulin levels were determined by radioimmunoassay using porcine insulin as a standard.

The glucose uptake (Gu) by the peripheral tissue was equal to isotopically determined Gd, unless glucose infusion rate (GIR) during the last 20 min of clamp was less than Gd. If GIR was higher than Gd, Gu was adopted to GIR. The hepatic glucose output (HGO) was calculated by the equation; $HGO = Gd - GIR$, unless GIR was less than Gd. If GIR was higher than Gd, HGO was determined to be zero, because HGO was estimated to be completely suppressed by the exogenous insulin. Then the percent suppression of HGO was calculated according to Moxley et al. (1990); percent suppression of $HGO = (1 - HGO_{clamp}/HGO_{basal}) \times 100$ (%).

Data were expressed as mean $\pm$ S.D. Statistical comparison was done by a non-parametric method, Mann-Whitney's U-test, with p-value less than 0.05 as significant.

Results

Changes in food intake, body weight, urine volume, urinary glucose excretion, and fasting plasma glucose level after AO-128 treatment

As shown in Fig. 1, food intake and body weight gain of Wistar fatty rats with and without AO-128 were not different. However, the urine volume of AO-128 treated rats at the 28th day was 27 ± 6 ml/day, significantly lower than 51 ± 18 ml/day of the rats without AO-128. Urinary glucose excretion of Wistar fatty rats was also decreased to 0.5 ± 0.5 g/day by AO-128 treatment, significantly lower than 3.2 ± 1.6 g/day of the control. Plasma glucose level at 14 : 00 to 16 : 00 hr of Wistar fatty rats also significantly decreased and reached the normal level, 126 ± 17 mg/100 ml, by AO-128 treatment (vs. 226 ± 72 mg/100 ml of Wistar fatty rats without AO-128 treatment).

Glucose infusion rate during clamp of Wistar fatty rats treated with AO-128

As shown in Fig. 2, GIR during the last 20 min clamp steady state of Wistar fatty rats with AO-128 treatment was 3.96 ± 1.47 mg$\cdot$kg$^{-1}\cdot$min^{-1}, higher than that of Wistar fatty rats without AO-128 treatment (1.30 ± 1.01 mg$\cdot$kg$^{-1}\cdot$min^{-1}), although GIR of both groups of Wistar fatty rats was extremely lower than that of lean littermates, 25.79 ± 5.13 mg$\cdot$kg$^{-1}\cdot$min^{-1}, indicating the presence of the insulin resistance in both Wistar fatty rats even after treatment with AO-128. We tried to examine the dose-response relationship between exogenous infused insulin and GIR, but the blood glucose levels did not decrease to 140 mg/100 ml when the lower dose of insulin was infused. When 20 mU$\cdot$kg$^{-1}\cdot$min^{-1} insulin

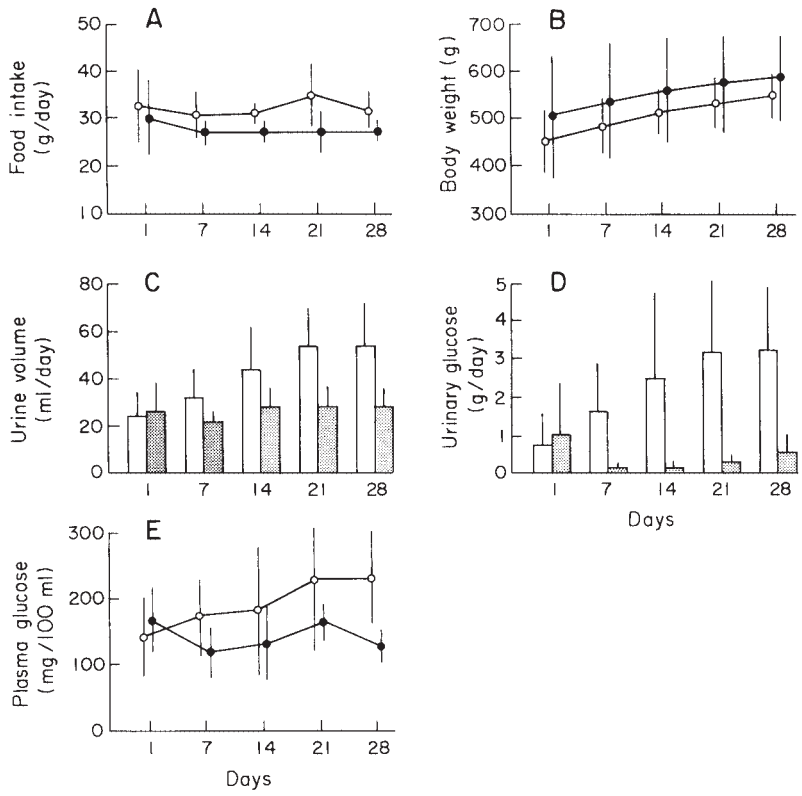

Fig. 1. Changes in food intake (A), body weight (B), urine volume (C), urinary glucose excretion (D), plasma glucose levels (E) in Wistar fatty rats with and without AO-128 treatment.
The open circle (○) and open column (□) represent data of Wistar fatty rats without AO-128 treatment, and the closed circle (●) and closed column (▩) represent data of AO-128 treated Wistar fatty rats. Plasma glucose levels were measured between 14 : 00 and 16 : 00 hr.

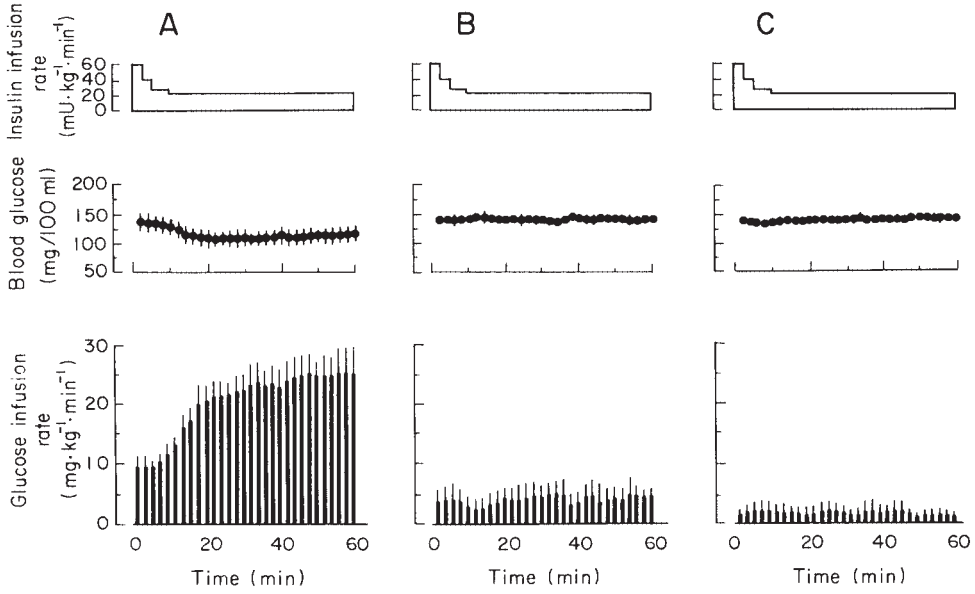

Fig. 2. Glucose clamp of lean littermates (A, $n=6$), Wistar fatty rats treated with AO-128 (B, $n=6$), and Wistar fatty rats without AO-128 treatment (C, $n=6$).

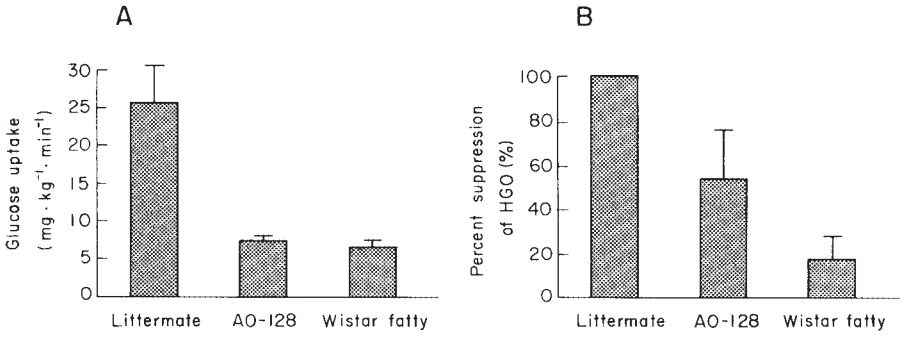

Fig. 3. Glucose uptake (A) and percent suppression of hepatic glucose output (B) during clamp steady state.

was infused, the peripheral level of insulin was $1762.0 \pm 433.5\ \mu U/ml$, and the stimulation of insulin effect was the maximum, because GIR during clamp with higher dose of insulin infusion (50 and 200 $mU \cdot kg^{-1} \cdot min^{-1}$) was not higher than that with 20 $mU \cdot kg^{-1} \cdot min^{-1}$ (data are not shown). We could examine and compare GIR during the clamp with only one dose of insulin infusion, 20 $mU \cdot kg^{-1} \cdot min^{-1}$.

Glucose uptake by peripheral tissue and percent suppression of hepatic glucose output during clamp steady state

As shown in Fig. 3A, Gu of both groups of Wistar fatty rats with and without AO-128 treatment (7.60 ± 0.63 $mg \cdot kg^{-1} \cdot min^{-1}$ and 6.64 0.91 $mg \cdot kg^{-1} \cdot min^{-1}$, respectively) was lower than that of lean littermates (25.79 ± 5.13 $mg \cdot kg^{-1} \cdot min^{-1}$). There was no significant difference in Gu of Wistar fatty rats with and without AO-128 treatment. However, as shown in Fig. 3B, percent suppression of HGO of Wistar fatty rats treated with AO-128 was $52.0 \pm 22.7\%$, which did not reach the complete (100%) suppression as with normal lean littermates, but it was significantly higher than that of Wistar fatty rats without AO-128 treatment ($17.4 \pm 11.2\%$).

Discussion

This study clearly showed that AO-128 treatment improved insulin resistance of Wistar fatty rats, but that the extent of improvement was only slight. Friedman et al. (1991) reported that the decreased levels of GLUT 4 of fatty Zucker rats were normalized by the treatment with a disaccharidase inhibitor, acarbose. Since GLUT 4 level is the rate-limiting step of insulin stimulated glucose disposal, normalization of GLUT 4 level might considerably improve in vivo insulin resistance of fatty Zucker rats. The difference in the extent of improvement between the Friedman's observation and our present investigation seemed to be due to different strains. Peripheral insulin levels of Wistar fatty rats are extremely higher than fatty Zucker rats (fa/fa) (Sugiyama et al. 1989). On the other hand, Shinozaki et al. (1996) have been reported that the SSPG of

patients with hyperinsulinemia significantly decreased after AO-128 treatment, but did not reach the level of the normal control. The results of the incomplete recovery of insulin resistance in this observation was in accord with our present study.

It is hardly thought that a disaccharidase inhibitor by itself corrects the abnormality of post-receptor pathway of both the peripheral tissue and liver in insulin resistant diabetic rats. We do not think that the amelioration of the glycemic control of Wistar fatty rats treated with AO-128 was caused by the slight improvement of insulin resistance, confirmed by glucose clamp technique. The reverse might be true. Indeed, it has been reported that the normalization of the glucose level of type I diabetic patients resulted in an improvement of sensitivity of the whole body to insulin (Vourinen-Markkola et al. 1992). Therefore the improvement of glycemic control by the delayed absorption of glucose with a disaccharidase inhibitor is likely responsible for the slight improvement of insulin resistance.

We would like to propose the possibility of two components of insulin resistance; a primary or genetically determined, and a secondary or poor-glycemic control-related insulin resistance. Only the latter insulin resistance could be normalized by a disaccharidase inhibitor. Since the improvement of insulin resistance with a disaccharidase inhibitor was slight, the contribution of the poor-glycemic control-related insulin resistance might be low, compared to the genetically determined insulin resistance. On the other hand, because thiazolidinediones, a new type of antidiabetic drug, improved completely the insulin resistance of Wistar fatty rats (Ikeda et al. 1990) as well as the insulin resistance of insulin dependent diabetes mellitus (IDDM) model animal, streptozotocin-induced diabetic rats (Tominaga et al. 1993), this drug could improve both components of insulin resistance. We also found that intensified insulin treatment, enough to achieve the normalized glycemic control, completely improved the insulin resistance of streptozotocin-induced diabetic rats (Tominaga et al. 1993).

Details in different molecular abnormalities between the genetically determined and the poor-glycemic contrl-related components of insulin resistance are so far not clear. Understanding the mode of action of thiazolidinediones would help us to know the difference between the two components of insulin resistance. However, the mechanism of improvement of thiazolidinediones on insulin resistance is not understood fully, although thiazolidinediones have been reported to enhance the insulin stimulated PI_3 kinase activity which is reduced in insulin resistant state of NIDDM model animals (Zhang et al. 1994). By the recent observation of thiazolidinediones as a ligand of peroxisome proliferator-activated receptor (PPAR) γ (Lehmann et al. 1995), the molecular mechanisms of the insulin resistance and the molecular bases of action of thiazolidinediones are still unknown. Therefore, the difference between the two components of insulin

resistance needs to be elucidated more exactly.

In conclusion, in Wistar fatty rats, there might be a genetically determined and a poor-glycemic control-related components of insulin resistance, the latter of which was improved by a disaccharidase inhibitor.

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