Metformin Decreases Blood Pressure and Obesity in OLETF Rats Via Improvement of Insulin Resistance

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To determine whether improvement of insulin resistance decreases blood pressure as well as obesity, metformin (100 mg/kg/d) or vehicle was administered for 20 weeks to 12-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats (n=10 each), a newly developed animal model of non-insulin-dependent diabetes mellitus (NIDDM) with mild obesity, hyperinsulinemia, and hypertriglyceridemia. Oral administration of metformin ameliorated glucose intolerance and attenuated the insulin response to glucose loading (2 g/kg, i.p.), as evidenced by a decrease in the area under the curve for glucose and insulin at 24 weeks by 19% and 37%, respectively. At 21 weeks, systolic blood pressure was significantly lower in the metformin group than in controls (130 ± 1.9 vs. 143 ± 2.7 mmHg, p < 0.01), despite no difference in body weight. Subsequently, blood pressure tended to be slightly but insignificantly lower in the metformin group, and body weight was significantly lower in the metformin group (532 ± 9.8 vs. 587 ± 10.3 g at 31 weeks, p < 0.01). Metformin treatment also lowered the level of serum triglycerides (9.4 ± 0.6 vs. 13.2 ± 0.5 mmol/l, p < 0.01) and the plasma norepinephrine concentration (4,222 ± 373 vs. 7,548 ± 1,058 pg/ml, p < 0.01). These results suggest that metformin-induced improvement of insulin resistance in obese rats with NIDDM may lower blood pressure, as well as decrease sympathetic activity and reduce body weight. (Hypertens Res 1996; 19: 37-41)

Key Words: metformin, OLETF (Otsuka Long-Evans Tokushima Fatty), insulin

Essential hypertension has recently been shown to be often associated with obesity, glucose intolerance, or non-insulin-dependent diabetes mellitus (NIDDM) (1, 2). In fact, using the euglycemic hyperinsulinemic clamp technique, patients with essential hypertension, even if they are not obese, have been shown to have insulin resistance (3), which is a common feature of NIDDM and obesity. In this sense, hypertension, NIDDM, and obesity share the common pathophysiological characteristic of insulin resistance.

Metformin, a biguanide derivative, is now widely used for the management of NIDDM (4, 5). It has been suggested that metformin lowers the level of blood glucose through its extrapancreatic action, i.e., by facilitating glucose utilization in peripheral tissues such as skeletal muscle (4-8). Recently, metformin treatment has been demonstrated to potentiate insulin-induced translocation of the glucose transporters (GLUT) GLUT1, GLUT4, or both (6-8). If metformin ameliorates insulin resistance, it is likely that the agent would not only improve hyperglycemia and hypertriglyceridemia but also lower blood pressure. In fact, recent studies have shown that metformin decreased blood pressure in nine non-obese, non-diabetic, non-smoking, middle-aged men with untreated hypertension after 6 weeks of treatment with 850 mg/d (9) and in 12 obese, non-diabetic hypertensive women after 12 weeks of treatment with 850 mg/d (10), although another study revealed no effect of metformin on blood pressure (11). The present study was therefore designed to determine the effects of long-term administration of metformin on body weight, blood pressure, glucose tolerance, and hypertriglyceridemia in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are a new animal model of NIDDM associated with mild obesity, hyperinsulinemia, and hypertriglyceridemia, established by Kawano et al. (Otsuka Pharmaceutical Co., Tokushima, Japan) in 1992 (12).

Materials and Methods
Metformin (kindly donated by Sumitomo Pharmaceutical Co., Osaka, Japan) was administered orally at 100 mg/kg/d for 20 weeks to male, 12-week-old OLETF rats (n=10), which were obtained from Otsuka Pharmaceutical Co. Controls (n=10)

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received tap water alone. This dose was selected since it has been proven to have a sufficient hypoglycemic effect (13).

Body weight was recorded every other week, and blood pressure was determined by the tail-cuff method every month. Fasting blood glucose levels were measured every two to five weeks using blood samples obtained from a tail vein. Plasma glucose and insulin levels were determined before and 60 and 120 min after intraperitoneal glucose loading at 2 g/kg, which was performed at 12, 24, and 32 weeks. Intraperitoneal glucose loading has been proven to be useful in evaluating the degree of hyperglycemia in spontaneously hypertensive rats (14) as well as in fructose-fed rats (15) in our laboratory. Plasma triglyceride and catecholamine levels were determined at 32 weeks of age after an overnight fast. Blood or plasma glucose levels were determined by a standard glucose oxidase method and insulin concentration by a specific radioimmunoassay (Phadesef Insulin kit, Pharmacia, Sweden) using rat insulin as a standard. Catecholamine levels were determined after separation by HPLC.

The gastrocnemius muscle was obtained from rats at 32 weeks of age. Gene expression and the plasma membrane protein concentration of GLUT4 were determined by Northern and Western blot analysis, respectively. The procedures used for Northern and Western analysis have been described previously (16, 17). In brief, total RNA was extracted from the muscle by the AGPC method (18) and size-separated through 1.2% agarose gel. The gel was blotted to a nitrocellulose membrane, which was then baked at 80°C in a vacuum oven for 2 h. The blot was hybridized to rat GLUT4 cDNA labelled with 32P by the random-primer method. After hybridization, the blots were washed, dried and then exposed to X-ray film (Fuji HR, Fuji Medical System Co., Ltd., Tokyo, Japan). Autoradiographs were scanned with a Shimazu densitometer (Tokyo, Japan). The DraI-Xba fragment of rat GLUT4 cDNA (nucleotide 143-1947) (17) and alpha-actin cDNA (obtained from Oncor Gene, Gaithersberg, MD) were used as probes. For Western analysis, muscles were homogenized in 10 mM Tris-HCl, 1 mM EDTA and 250 mM sucrose, pH 7.4, containing 1 mM phenylmethylsulfonyl fluoride and 1,000 U/ml aprotinin. The homogenates were centrifuged at 700 × g for 10 min at 4°C to sediment the fraction containing the nuclei and mitochondria. The supernatant was centrifuged at 12,000 × g for 15 min at 4°C. The resulting supernatant was centrifuged at 146,000 × g for 75 min at 4°C to obtain the membrane fraction. Membrane fractions were suspended in 1% SDS and 50 mM dithiothreitol and subjected to SDS-polyacrylamide (10%) gel electrophoresis as described by Laemmli (19). Electrophoretic transfer to nitrocellulose membrane and detection of the immunocomplex with 125I-labeled protein A (Amersham, Amersham, UK) were carried out with antiserum against GLUT4 at a final dilution of 1: 20 (17). The dried blots were autoradiographed as described above.

Statistical Analysis
All data were expressed as the mean ± SEM.
Differences were analyzed by paired or unpaired Student's t test and were considered to be significant at p<0.05.

Results
Changes in body weight and blood pressure are shown in Fig. 1. Metformin treatment attenuated body weight gain significantly after 27 weeks. Blood pressure in controls increased significantly with age, reaching about 160 mmHg at 23 weeks. At 21 weeks, blood pressure in the metformin group was

Fig. 1. Changes in body weight and blood pressure in the metformin group (solid circles) and controls (open circles). Values are expressed as means ± SEM.

Fig. 2. Changes in fasting blood glucose levels in the metformin group (solid circles) and controls (open circles).
significantly lower than that in the control group, although there was no difference in body weight between these groups. After 21 weeks, blood pressure in the metformin group was about 10 mmHg lower than in the control group, although the difference was not statistically significant.

Fasting blood glucose levels are shown in Fig. 2. In the control group, fasting blood glucose concentration increased with age, reaching about 7.8 mmol/l at 15 weeks and 10.0 mmol/l at 22 weeks. Metformin produced a slight, but not significant, decrease in the level of blood glucose between 27 and 30 weeks. Figure 3 shows the results of the i.p. glucose tolerance test. Metformin improved glucose tolerance significantly as indicated by lower blood glucose levels after glucose loading at both 24 and 32 weeks. At 24 weeks, plasma insulin levels were significantly lower at fasting and 60 min after glucose loading. At 32 weeks, the fasting serum insulin concentration reached about 10.2 to 12.0 pmol/l, which was significantly higher than that at 24 weeks. However, there was no difference between the control and the metformin groups. The area under the curve (AUC) for plasma glucose and the insulin response to glucose loading at 12, 24, and 32 weeks are shown in Table 1. The AUC for plasma glucose levels in the treated group was lower than in controls at both 24 and 32 weeks. Metformin treatment also decreased the AUC for insulin concentration at 24 weeks, but not at 32 weeks. In addition, metformin decreased the serum triglyceride level significantly to 9.4 ± 0.6 mmol/l at 32 weeks (vs. 13.2 ± 0.5, p<0.05).

A representative Northern blot for skeletal muscle GLUT4 mRNA is illustrated in Fig. 4. Metformin increased the gene expression of GLUT4 slightly, but not significantly. A representative Western blot for skeletal muscle GLUT4 is also illustrated in Fig. 4. Metformin-treated animals showed a two-fold increase in staining with the specific antibody.

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**Table 1. Area under the Curve (AUC) of Glucose and Insulin during GTT (2g/kg, i.p.) at 12, 24, and 32 Weeks of Age**

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<th>12</th>
<th>24</th>
<th>32</th>
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<tr>
<td>AUC Glucose (mmol/l×h)</td>
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<tr>
<td>Metformin</td>
<td>20.4±1.7</td>
<td>21.5±0.9</td>
<td>26.6±2.9</td>
</tr>
<tr>
<td>Control</td>
<td>19.4±1.0</td>
<td>26.5±2.0</td>
<td>40.8±2.1</td>
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<tr>
<td>AUC Insulin (pmol/l×h)</td>
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</tr>
<tr>
<td>Metformin</td>
<td>14.4±2.4</td>
<td>7.2±0.6</td>
<td>24.6±3.6</td>
</tr>
<tr>
<td>Control</td>
<td>13.2±1.8</td>
<td>11.4±1.8</td>
<td>27.0±2.4</td>
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mean±SEM. a: p<0.05 vs. Control; b: p<0.01 vs. Control; c: p<0.05 vs. 12 weeks; d: p<0.01 vs. 12 weeks.

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![Graphs showing glucose and insulin levels](image-url)
against GLUT4.

Plasma catecholamine levels are illustrated in Table 2. Metformin administration decreased the levels of plasma norepinephrine and dopamine significantly.

Discussion

In the present study, metformin clearly improved glucose tolerance, as evidenced by lower fasting plasma glucose levels and lower glucose levels in response to i.p. glucose loading. Triglyceride levels were also decreased by metformin treatment. Although the plasma insulin response to glucose loading was not lowered significantly, these results indicate that metformin may improve peripheral insulin resistance. This was supported by the observation that the GLUT4 protein content of muscle plasma membrane was augmented, consistent with previous studies demonstrating an increase in insulin-induced GLUT4 translocation by metformin administration (6-8).

Of interest was the observation that metformin treatment decreased not only body weight but also blood pressure. In fact, body weight reduction after metformin administration has been reported in some studies (20, 21), but not all (9-11). This is in contrast with the weight gain observed in patients treated with sulfonylureas. Metformin has been reported to decrease appetite, resulting in reduced food intake (22). Although we did not measure the amount of food intake in the present study, food intake did not differ between controls and metformin-treated OLETF rats in another study (unpublished observation). The lower body weight gain in the metformin group may therefore be attributable to improved insulin resistance, resulting in greater energy expenditure of glucose, lipids, or both. Further studies are needed to establish that weight gain is a consequence of insulin resistance, even if they are often associated, since good insulin sensitivity, not insulin resistance, in humans is a predictor of weight gain. Nonetheless, the decrease in blood pressure in the treated group may have been due to a decrease in body weight. However, it should be noted that metformin lowered blood pressure by about 20 mmHg as early as 21 weeks of age when body weight did not differ from that of controls. This finding supports previous studies in humans (9, 10). Improved insulin resistance may contribute to a decrease in blood pressure. In addition, plasma catecholamine levels in the metformin group were significantly lower than in controls (9), which is consistent with a previous study in humans (10), and this may be another reason why metformin decreased blood pressure. Although we did not find a significant decrease in plasma insulin response during glucose tolerance tests at 24 and 32 weeks of age despite an improved glucose tolerance, plasma insulin levels before and 60 min after glucose loading at 24 weeks were significantly lower in the group receiving metformin. Hyperinsulinemia has been shown to increase plasma catecholamine levels and muscle sympathetic nerve activity in the absence of hypoglycemia (23-25). Metformin-induced amelioration of insulin resistance may cause a decrease in plasma insulin levels, resulting in a lowering of catecholamine levels. Such a blood pressure decrease has been reported in obese Zucker rats and obese rhesus monkeys treated with the newly developed hypoglycemic agents troglitazone, ciglitazone, and pioglitazone, which improve insulin sensitivity (26-28), although we did not observe a blood pressure decrease in spontaneously hypertensive rats (SHR) treated with troglitazone (14).

In contrast, catecholamine causes a 40-50% de-

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<th>Table 2. Plasma Catecholamine Concentrations in the Control and Metformin Groups</th>
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<td>Norepinephrine (pg/ml)</td>
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<td>Epinephrine (pg/ml)</td>
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<td>Dopamine (pg/ml)</td>
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mean±SEM, **p<0.01 vs. control.
crease in the rate of whole-body glucose uptake (29), i.e., insulin resistance, possibly through α2-adrenergic receptors (30). The decrease in catecholamine levels observed in OLETF rats treated with metformin may ameliorate insulin resistance.

Although the exact mechanism of action remains to be defined, metformin may be a unique hypoglycemic agent that lowers not only hyperglycemia but also hypertriglyceridemia, and high blood pressure in this rodent NIDDM model. Perhaps by improving insulin resistance, the drug may have similar effects in humans with NIDDM associated with mild obesity.

References

14. Katayama S, Abe M, Kashiba H, Kosegawa I, Ishii J: Evidence against a role of insulin in hyperten-