The three major pathophysiology of type 2 diabetes are peripheral insulin resistance, increased hepatic glucose production, and impaired insulin secretion [1]. Insulin resistance is a hallmark of the development...
of type 2 diabetes [2]. In addition, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease are associated with insulin resistance [1]. Improving insulin resistance prevents hyperglycemia and may reduce cardiovascular events.

Metformin and troglitazone are oral antidiabetic agents used to reduce insulin resistance. Metformin is a biguanide molecule that has been used for more than 30 years for the treatment of type 2 diabetes. It acts primarily by inhibiting hepatic glucose production and increasing the sensitivity of peripheral tissues to insulin [3–5]. Metformin also has beneficial effects on lipid metabolism [6, 7] and reduces blood pressure in hypertensive, obese patients [7]. Troglitazone is a member of the thiazolidinediones, a new class of oral antidiabetic agents. It has been shown to reduce hyperinsulinemia, hypertriglyceridemia, and hepatic glucose production and mainly improves peripheral glucose utilization without stimulation of insulin secretion [4, 8–10].

Rodent models of insulin resistance show that a high-fructose diet results in hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and hypertension [9, 11]. This animal model resembles certain features of the non-diabetic insulin-resistant states in humans [12]. The aim of this study was to compare the metabolic effects of metformin and troglitazone in fructose-induced insulin-resistant rodents.

Materials and Methods

Male Sprague-Dawley rats weighing 150–200 g were housed two per cage in an environmentally controlled room with a 12-hour light/dark cycle (light 0700–1900 hours) and had free access to food and water.

After a 7-day acclimation period, the rats were fed ad libitum and allocated to receive one of the following four treatments for 6 weeks: normal rat chow (powdered rodent diet 8604; Harlan Tekland, Madison, WI, USA) (control group, n = 7); high-fructose diet (TD 89247; Harlan Tekland) in which the fructose provided 60% of total calories (fructose group, n = 7); high-fructose diet plus metformin (metformin group, n = 8); and high-fructose diet plus troglitazone (troglitazone group, n = 8). Metformin and troglitazone were given as 0.75% and 0.2% food admixtures, respectively.

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Animal Subjects Committee of China Medical College.

Blood pressure was measured before the start of the study and again after 6 weeks. Each rat was warmed for 15 minutes in a preheated box at 40°C, then moved to an acrylic box for immobilization. Systolic blood pressure of the tail region was measured using a volume-oscillometric method (UR-500, Ueda, Tokyo, Japan) [13].

Food was removed at 0800 hours. Blood samples were obtained from the jugular vein at 1300 hours and centrifuged within 30 minutes of collection. Serum samples were stored at –70°C for later measurement. Serum insulin concentrations were measured using an enzymatic immunoassay (Mercodia rat insulin, Mercodia AB, Uppsala, Sweden), triglyceride concentrations using an enzymatic colorimetric method (Toshiba-80 FR, Tokyo, Japan), free fatty acid (FFA) concentrations using an enzymatic colorimetric method (NEFA, Boehringer Mannheim, Indianapolis, IN, USA), and leptin concentrations using a radioimmunoassay (Linco Research, St. Louis, MO, USA).

Statistical analysis

All data are presented as means ± standard deviations. Statistical analyses were performed using analysis of variance (ANOVA) and Tukey’s test. Analysis of covariance (ANOCOVA) was used to examine the mean differences among groups after adjusting basal values. A p value of less than 0.05 was considered statistically significant.

Results

Because the rats were housed two per cage, only the average food intake could be estimated. Food intakes were 23 g/day/rat in the control group, 22 g/day/rat in the fructose group, 20 g/day/rat in the metformin group, and 22 g/day/rat in the troglitazone group.

There were no significant differences in basal and final body weight between the groups (Table). Nonetheless, after adjusting for basal body weight, the metformin group had significantly less weight gain than the other three groups.

There was a significant difference in basal systolic blood pressure (SBP) between the metformin group and troglitazone group (127.9 ± 7.1 vs 138.6 ± 5.5 mm Hg, p < 0.05). The high-fructose diet led to significantly higher SBP (152.4 ± 13 vs 138.1 ± 7.5 mm Hg, p < 0.05). Troglitazone, but not metformin, significantly lowered fructose-induced high SBP (137.8 ± 9.2 vs 152.4 ± 13 mm Hg, p < 0.05). After adjusting for basal SBP, the final SBP in the metformin group was significantly higher than that in the troglitazone group (147.8 ± 5.8 vs 137.8 ± 9.2 mm Hg, p < 0.05) (Table).

After 6 weeks of high-fructose diet, rats developed significant hyperinsulinemia (48.4 ± 15.2 vs 22.2 ± 10.4 mU/µL, p < 0.05). Metformin and troglitazone

Table. Metabolic effects of the four diets after 6 weeks

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 7)</th>
<th>Fructose group (n = 7)</th>
<th>Metformin group (n = 8)</th>
<th>Troglitazone group (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBW (g)</td>
<td>258.2 ± 39.5</td>
<td>252.1 ± 49.1</td>
<td>260 ± 40.5</td>
<td>250.2 ± 33.3</td>
</tr>
<tr>
<td>FBW (g)*</td>
<td>470.5 ± 51.8</td>
<td>470.1 ± 59.6</td>
<td>397.9 ± 40.9†</td>
<td>452.5 ± 32.8§</td>
</tr>
<tr>
<td>BSBP (mmHg)</td>
<td>135.7 ± 6.4</td>
<td>133.7 ± 9.4</td>
<td>127.9 ± 7.1</td>
<td>138.6 ± 5.5§</td>
</tr>
<tr>
<td>FSBP (mmHg)*</td>
<td>138.1 ± 7.5</td>
<td>152.4 ± 13.0†</td>
<td>147.8 ± 5.8†</td>
<td>137.8 ± 9.2§</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>22.2 ± 10.4</td>
<td>48.4 ± 15.2†</td>
<td>14.8 ± 10.5†</td>
<td>15.0 ± 13.6†</td>
</tr>
<tr>
<td>FFA (mg/dL)</td>
<td>47.0 ± 27.8</td>
<td>78.7 ± 24.6</td>
<td>45.9 ± 17.6</td>
<td>38.9 ± 22.7†</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>91.0 ± 40.9</td>
<td>250.1 ± 95.7†</td>
<td>75.3 ± 65.5†</td>
<td>67.6 ± 32.4†</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>7.7 ± 2.2</td>
<td>6.9 ± 2.0</td>
<td>3.1 ± 1.5†</td>
<td>4.4 ± 2.0††</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation; BBW = basal body weight; FBW = final body weight; BSBP = basal systolic blood pressure; FSBP = final systolic blood pressure; FFA = free fatty acid; TG = triglyceride; †p < 0.05 vs control group; ‡p < 0.05 vs fructose group; §p < 0.05 vs metformin group. *Statistical analysis of the mean differences among groups was performed after adjusting basal values.

Significantly reduced fructose-induced hyperinsulinemia (14.8 ± 10.5 vs 48.4 ± 15.2 µU/mL, p < 0.05; 15.0 ± 13.6 vs 48.4 ± 15.2 µU/mL, p < 0.05, respectively). There was no significant difference in serum insulin concentrations between the metformin group and the troglitazone group (14.8 ± 10.5 vs 15 ± 13.6 µU/mL, p > 0.05) (Table).

The mean serum FFA concentration was highest in the fructose group, but the difference was not statistically significant. Fructose-induced high FFA concentrations were significantly reduced in the troglitazone group but not in the metformin group. The difference in serum FFA concentrations between the metformin group and the troglitazone group was not significant (45.9 ± 17.6 vs 38.9 ± 22.7 mg/dL, p > 0.05) (Table).

Rats on a high-fructose diet developed significant hypertriglyceridemia (250.1 ± 95.7 vs 91.0 ± 40.9 mg/dL, p < 0.05). Metformin and troglitazone significantly reduced fructose-induced hypertriglyceridemia. There was no significant difference in serum triglyceride concentrations between the metformin group and the troglitazone group (75.3 ± 65.5 vs 67.6 ± 32.4 mg/dL, p > 0.05) (Table).

Leptin concentrations were not significantly different between the fructose group and the control group (6.9 ± 2.0 vs 7.7 ± 2.2 mg/mL, p > 0.05). Metformin and troglitazone significantly reduced serum leptin concentrations. There was no significant difference in serum leptin concentrations between the metformin group and the troglitazone group (3.1 ± 1.5 vs 4.4 ± 2.0 ng/mL, p > 0.05) (Table). After adjusting for final body weight, the results remained unchanged.

Discussion

In this study, administration of a high-fructose diet to normal rats resulted in insulin resistance with elevated SBP and FFA concentration, hyperinsulinemia, and hypertriglyceridemia.

Metformin is widely used in the treatment of type 2 diabetes, especially for obese patients. It lowers body weight [5, 10, 14–16], in part by reducing food intake [10, 15]. Because the rats were housed two per cage, strict statistical comparison of food intake between groups was not possible. However, our data revealed that metformin-fed rats consumed less food and gained less weight than the rats in the other three groups. The body weight-reducing effect also contributed to improvement in insulin resistance [17].

Recent studies have demonstrated that troglitazone combined with diet or glibeclamide for 12 weeks increases hunger and promotes weight gain [18]. Significant weight gain was observed when troglitazone (400 mg/day) was added to sulfonylureas for 12 weeks [19], and significant dose-dependent weight gain was observed when troglitazone was added to glyburide (12 mg/day) [20]. In contrast, Kelly et al reported that treatment with troglitazone alone at a dose of 600 mg/day did not affect body weight of patients with type 2 diabetes [21]. Our study also showed no significant weight gain in troglitazone-fed rats. This discrepancy may be due to the combination with sulfonylurea; the United Kingdom Prospective Diabetes Study revealed that sulfonylurea treatment results in significant increases in body weight when compared with dietary or metformin treatment [22].

Considine et al found a strong positive correlation between serum leptin concentration and percentage of body fat [23]. After 6 weeks, leptin concentration was significantly reduced to 3.1 ± 1.5 ng/mL in the metformin group and 4.4 ± 2.0 ng/mL in the troglitazone group. The leptin-lowering effect of metformin and troglitazone was independent of body weight. Okuno et al showed that troglitazone does not change the total weight of white adipose tissue in obese

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rats but increases the number of small adipocytes and decreases the number of large adipocytes. These changes are associated with lower amounts of leptin [24]. Altering the size of adipocytes but not their total weight may explain why the leptin-lowering effect of troglitazone is independent of body weight. Paolisso et al demonstrated that metformin administration decreased plasma leptin concentration in obese subjects, which was mainly due to a decline in body fat content [15]. Our study revealed that leptin concentration in metformin-fed rats was independent of body weight. Further investigation is needed to determine other possible mechanisms for the leptin-lowering effect of these two drugs.

It has been suggested that insulin resistance and/or hyperinsulinemia leads to hypertension. Increasing insulin sensitivity and/or reversal of hyperinsulinemia results in a concomitant reduction in blood pressure [12]. Inzucchi et al showed that metformin acts primarily by decreasing endogenous glucose production and troglitazone by increasing the peripheral glucose disposal rate [4]. Our study revealed that the troglitazone group, but not the metformin group, had significantly lower SBP compared to the control group. Perhaps the different mechanisms of action cause different SBP-reducing effects.

Our study confirmed previous findings that both metformin and troglitazone reduce hyperinsulinemia and hypertriglyceridemia [7–9]. The insulin and triglyceride concentrations were not significantly different between the metformin and troglitazone groups in this study. Because the fructose group did not develop significantly higher FFA concentrations, only troglitazone significantly lowered the FFA concentration. Nonetheless, there was no significant difference in the FFA concentration between the metformin group and the troglitazone group.

In conclusion, a high-fructose diet for 6 weeks led to insulin resistance in normal rats. Both metformin and troglitazone were comparably effective in improving this insulin resistance. Metformin had the advantage of body weight reduction but was not effective in reducing SBP. Troglitazone had the advantage of lowering SBP but did not reduce body weight. The different unique effects of metformin and troglitazone on body weight and SBP facilitate their selection in different conditions of type 2 diabetes. In patients with obese normotensive type 2 diabetes, metformin is the drug of choice [25], while in hypertensive type 2 diabetic patients, troglitazone may be a better choice than metformin. Both metformin and troglitazone reduced leptin concentrations in our study. Further investigation is needed to elucidate the mechanism(s) of the leptin-lowering effect of these two drugs.

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References