Biotin Administration Improves the Impaired Glucose Tolerance of Streptozotocin-Induced Diabetic Wistar Rats

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Summary The effect of biotin administration on the glucose tolerance of Streptozotocin (STZ)-induced diabetic Wistar rats was investigated. STZ-induced diabetes was induced by intraperitoneal injection of streptozotocin (45 mg/kg body weight as a single dose). The impaired glucose tolerance in response to an oral glucose load (1.8 g per kg body weight) in STZ-induced diabetic rats (STZ-rat) was partially improved by intraperitoneal administration of biotin for 15 days (100 µg/rat/day). However, a recovery in the STZ-rat’s insulin secretion was not found after biotin administration. To help clarify the mechanism underlying the improvement in glucose tolerance seen with biotin treatment, glucokinase and hexokinase activities were determined in the liver and pancreas. In STZ-rats that had received biotin (STZ-biotin rats), glucokinase activity was higher by 3.4-fold in liver and by 2.4-fold in pancreas than in the STZ-rats. The biotin level of STZ-rats was significantly lower in the liver and pancreas than that of the control rats (no STZ administration); but in STZ-biotin rats, the level in these organs recovered to the control level. These results demonstrate that injected biotin can improve glucose handling without increasing insulin secretion in STZ-rats.

Key Words biotin, streptozotocin (STZ)-induced diabetic rats, glucose tolerance test, glucokinase, IDDM (insulin-dependent diabetes mellitus)

It has been known for many years that biotin deficiency results in an impairment of glucose utilization (1–5). In our previous study, insulin secretion in response to an oral glucose load in biotin-deficient male Wistar rats was approximately one-sixth less, in concentration terms, than that of pair-fed control rats. However, the insulin content in the pancreas of the same biotin-deficient rats was no lower than that of the control rats. The impaired insulin secretion seen in these

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biotin-deficient rats was significantly improved by the simultaneous administration of biotin (1 mg per kg body weight) with the glucose solution (6). In addition, we recently found that the feeding of a high-biotin diet corrects impaired glucose tolerance (IGT) and the hyperinsulinemia of OLETF rats (a spontaneously diabetic model with NIDDM) (7). Furthermore, Coggeshall et al. (8) have demonstrated that a pharmacologic dose of biotin (16 mg/day for one week) lowers the fasting blood glucose concentration in insulin-dependent diabetic patients during insulin withdrawal. Subsequently, we demonstrated the therapeutic effectiveness of biotin in patients with non-insulin-dependent diabetic mellitus (NIDDM) (9). Taken all together, this indicates that the administration of high concentrations of biotin may improve the metabolism and/or utilization of glucose without accelerating the secretion of insulin from the pancreas. To clarify the mechanism underlying the effect of biotin on glucose metabolism in the absence of insulin, and assess the therapeutic effectiveness of biotin in diabetic rats with insulin-dependent diabetes mellitus (IDDM), we used Wistar rats as a model of IDDM, in which insulinomas were induced by streptozotocin-maintained hyperglycemia. In these rats, the islets of Langerhans are destroyed, so that insulin can not be secreted.

The aim of this study was to establish whether or not marginal biotin deficiency is one of the factors contributing to impaired glucose tolerance in insulin-dependent diabetic rats. In these experiments, we used IDDM rats that were receiving no insulin therapy. In terms of the rapid course of their diabetes mellitus, such STZ-Wistar rats resemble humans with IDDM (10).

**MATERIALS AND METHODS**

Animal, diet, and experimental design. Male Wistar rats, 4 weeks old and specifically pathogen-free, were obtained from Funabashi Farm (Tokyo, Japan). Each rat was individually housed in a stainless-steel cage in an air-conditioned room (24–25°C) with 50% humidity. The room was illuminated from 08:00 to 20:00 h. These rats, weighing 70–80 g, were habituated by feeding a commercially available solid feed (Type F-2, Funabashi Farm) for the entire experimental period; the diet and distilled water being provided ad libitum. At 6 weeks of age, some of the rats were made diabetic by a single intraperitoneal administration of streptozotocin (Wako Pure Chemical Industries, Osaka, Japan). The streptozotocin (45 mg/kg body weight) was dissolved in 0.1 M citrate buffer, pH 4.0, immediately before injection. The STZ-rats were divided into two groups: biotin (100 µg/rat, ip injection) was given to one of the two groups each day. The other groups, STZ rats and control rats (no STZ), were injected with saline solution. The body weight of each group was measured once every week.

Glucose tolerance test. An oral glucose tolerance test (OGTT) was carried out on each rat on days 3, 9 and 15 after STZ injection. The OGTTs were performed by measurement of plasma glucose concentration, and the insulin secretion of each animal was measured without anesthesia three times over the 15-
day period. For this purpose, each animal was fasted for 24 h and the blood for glucose and insulin determination was collected from the tail vein before, and 30, 60 and 120 min after feeding 1.8 g/kg body weight of glucose via a gastric catheter. The plasma glucose levels were estimated by a standard glucose oxidase method (11) (Glucose C-II test Wako, Wako Pure Chemical Industries). The immuno-reactive insulin (IRI) of the plasma was determined with a Shionogi Insulin RIA kit (Shionogi, Osaka, Japan) employing the double-antibody method of radio-immunoassay (12). The diagnosis of diabetes mellitus involved the classification of the plasma glucose level during an OGTT into one of the following three types: (i) normal pattern, (ii) impaired glucose tolerance (IGT) pattern, or (iii) diabetes mellitus (DM) pattern (13).

_Determination of biotin concentration in the plasma._ The plasma biotin concentration was determined microbiologically by means of a procedure described previously (14), using the test organism of _Lactobacillus plantarum_ (ATCC 8014). Samples of plasma or organ homogenates (see below) were acid-hydrolyzed prior to the assay.

_Determination of glucokinase and hexokinase activities in the liver and pancreas._ After the OGTT on the 15th day, all rats were fasted overnight and anesthesized with diethylether. Blood was then collected from the abdominal aorta. After perfusion with saline, the liver and pancreas were removed and weighed for glucokinase and hexokinase activity. Tissue homogenates were prepared by 20 strokes of a kontes glass homogenizer. The homogenizing buffer was 50 mM NaHEPES, pH 7.8, containing 1 mM EDTA, 1 mM dithiothreitol and 110 mM KCl. Tissue homogenates were centrifuged at 105,000 × g for 60 min at 20°C. Glucokinase and hexokinase activities of the homogenate were measured using glucose concentrations between 5 and 100 mM, and measuring the rate of absorbance at 340 nm with a spectrophotometer (15).

_Statistical analysis._ All results are expressed as mean ± SE. A statistical analysis was performed by analysis of variance (ANOVA) coupled with Duncan’s multiple range test for classification of the means, with \( p < 0.05 \) accepted as the level of significance.

**RESULTS**

_Growth profiles_ The growth curves for the STZ, STZ-biotin and control Wistar rats are shown in Fig. 1. At the start of the experiment (6 weeks of age), the body weight of the rats was approximately 120 g. STZ and STZ-biotin rats grew less than the control rats. The body weights of the STZ and STZ-biotin groups were significantly less than that of the control rats at days 3, 6 and 15 after STZ injection. The average body weight of the STZ-biotin rats was not different from that of the STZ rats.
Fig. 1. Effect of biotin administration on the body weight of Wistar and Wistar-STZ rats. Wistar rats were made diabetic by injection of streptozotocin at 6 weeks old. The STZ-rats were divided into two groups, one of which (STZ-biotin) was given biotin (100 µg/rat/day, ip). Control group: (○), STZ group: (●), STZ-biotin group: (■). Results are expressed as mean ± SE. *p < 0.05, **p < 0.01 for comparison with control group (day 0 is equivalent to 6 weeks old).

Glucose tolerance and insulin secretion in response to oral glucose load

Figure 2 depicts the plasma glucose levels in STZ, STZ-biotin and control rats given 1.8 g glucose solution per kg of body weight to initiate each oral tolerance test. The STZ and STZ-biotin rats showed significantly higher plasma glucose levels than control rats before, 30, 60 and 120 min after glucose loading throughout the 15-day experimental period. Three days after STZ injection, the plasma glucose level in STZ-biotin rats (176 mg/dL) before glucose loading was significantly lower than the glucose level in STZ-rats (280 mg/dL). Moreover, the plasma glucose level in both STZ and STZ-biotin rats exceeded 400 mg/dL at 30 min and 300 mg/dL at 120 min. Thus, it was shown that they could be classified as having the diabetes mellitus (DM) pattern described in Materials and Methods (13). In the second OGTT (9th day), the fasting plasma glucose of STZ-rats showed an even higher glucose level than in the first OGTT, but the STZ-biotin rats exhibited a significantly lower glucose level than the STZ-rats both before and 30 min after glucose loading. In the third OGTT (15th day), the STZ-rats showed still lower values after glucose loading. Insulin levels
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Fig. 2. Effect of biotin on the plasma glucose and insulin responses to three glucose loads (3, 9 and 15 days after STZ injection). Control group: (○), STZ group: (●), STZ-biotin group: (■). Results are mean±SE (n=8). There are significant differences among the three groups at p<0.05 (between a and b, b and c, and a and c).

In both STZ and STZ-biotin rats failed to respond to glucose loading. Moreover, no difference in terms of insulin response was produced by the administration of biotin throughout the 15-day experimental period.

Organ weight and biotin content of plasma and organs

After STZ injection, the weight of the pancreas was significantly decreased in the STZ and STZ-biotin rats as compared to that of the control rats (Table 1). As shown in Fig. 3, the concentration of biotin in the liver of STZ-rats was significantly lower than that in the control rats. However, in the STZ-biotin group, the liver biotin level was not significantly lower than that in the control group. The biotin level in the pancreas of the STZ-rats was observed to be extremely lower than that in the control rats (Fig. 3). It is thought that STZ treatment affected the efficiency of the biotin metabolism. Although the biotin level in the pancreas of STZ-biotin rats was higher than that in STZ rats, there was not a significant difference. Throughout the 15-day experimental period, plasma biotin concentration in the STZ-rats was no lower than that in the control rats (Table 2). The concentra-
Table 1. Organ weights of Wistar and Wistar-STZ rats.

| Group      | Liver     | Pancreas  | Spleen   | Kidney    |
|------------|-----------|-----------|----------|-----------|
| Control    | 7.88±0.19 | 0.87±0.03 | 0.53±0.02| 1.63±0.04 |
| STZ        | 8.32±0.37 | 0.73±0.05 | 0.47±0.04| 1.76±0.06 |
| STZ+biotin | 7.79±0.24 | 0.73±0.03 | 0.50±0.03| 1.65±0.03 |

Values are mean±SE (n=8). Differences between three groups were analyzed by Duncan's multiple range test: values within a column with different superscript letters are significantly different from each other (p<0.05). Liver and pancreas were removed and weighed at 15 days after STZ injection.

Fig. 3. Effect of STZ and biotin administration on the concentration of biotin in the liver and pancreas. The biotin concentration in each group at 15 days after STZ injection is shown as mean±SE (n=8). Control group: (□), STZ group: (■), STZ-biotin group: (■). There are significant differences among the three groups at *p<0.05 or **p<0.01, as shown.

Table 2. Changes in the plasma concentration of biotin in Wistar and Wistar-STZ rats.

| Group      | Biotin concentration (ng/mL) |
|------------|------------------------------|
|            | 3-day | 9-day | 15-day |
| Control    | 4.81±0.18 | 4.60±0.07^a | 5.00±0.17^ab |
| STZ        | 4.73±0.11 | 4.69±0.13^ab | 4.62±0.11^a |
| STZ-biotin | 4.93±0.14 | 5.04±0.08^b | 5.15±0.14^b |

All rats were subsequently given a repeated oral glucose tolerance test (OGTT) during the 15-day experimental period. Values are mean±SE (n=8). The differences between the three groups were analyzed using a two-way ANOVA followed by Duncan's multiple range test: means in the same column not sharing a common superscript letter are significantly different (p<0.05).

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Table 3. Glucokinase and hexokinase activities in the liver and pancreas of Wistar and Wistar-STZ rats.

| Group    | Glucokinase (units/g tissue) | Hexokinase (units/g tissue) |
|----------|-----------------------------|-----------------------------|
|          | Liver                       | Pancreas                    | Liver                       | Pancreas                    |
| Control  | 0.33±0.11<sup>a</sup>       | 0.17±0.05<sup>a</sup>       | 0.35±0.16<sup>ab</sup>      | 0.15±0.06                   |
| STZ      | 0.39±0.09<sup>a</sup>       | 0.21±0.09<sup>a</sup>       | 0.28±0.04<sup>a</sup>       | 0.32±0.10                   |
| STZ-biotin | 1.34±0.26<sup>b</sup>   | 0.50±0.07<sup>b</sup>       | 0.66±0.07<sup>b</sup>       | 0.51±0.14                   |

The activities of glucokinase and hexokinase were determined by the procedures developed by Vinuela et al. (29). Values are mean±SE (n=5). The differences between the three groups were analyzed using a two-way ANOVA followed by Duncan’s multiple range test: means in the same column not sharing a common superscript letter are significantly different (p<0.05). Liver and pancreas were removed for determination of glucokinase and hexokinase activities at 15 days after STZ injection.

Activity of glucokinase and hexokinase in the liver and pancreas

Glucokinase activity in the STZ-biotin rats was 3.4-fold higher in the liver and 2.4-fold higher in the pancreas than that in STZ-rats, although there was no significant difference in the activities between the STZ and control rats (Table 3). Moreover, the STZ-biotin rats showed higher hexokinase activity in the liver than that in STZ rats. However, hexokinase activity in the pancreas showed no significant difference between the three groups.

DISCUSSION

This study demonstrated that biotin administration reduced the hyperglycemia seen in rats with IDDM but did not affect the secretion of insulin. The study also demonstrated a low biotin concentration in the liver and pancreas of STZ rats, which returned to the control level after biotin administration. These results indicate that the administration of high concentrations of biotin can improve the metabolism and/or utilization of glucose without accelerating the secretion of insulin from the pancreas. This phenomenon is similar to that seen in IDDM patients. Indeed, when the same parameters were measured in IDDM patients who had been removed from insulin therapy and had biotin treatment (16 mg/day) for one week, the subjects treated with placebo showed the expected elevation in the fasting blood sugar (FBS) level, while the FBS level in the biotin-treated subjects decreased significantly (8).

STZ-biotin rats had significantly higher glucokinase activity than the STZ and control rats (Table 3). This result suggests a biotin-enhancing effect on the
synthesis of glucokinase at the stage of translation in rat liver and pancreas as shown by Vesely and Mistry (16). Li Hsieh and Mistry (17) have also pointed out that glucokinase activity is low in diabetic, fasting biotin-deficient rats, and that de novo synthesis of the enzyme can be induced by insulin and biotin in rats. The activation of glucokinase in pancreatic \( \beta \)-cells may be an important reaction in the pathway leading to insulin secretion (18–22). The effects of biotin upon the intracellular level of cGMP and on the activity of glucokinase have been examined in primary cultures of adult rat hepatocyte: the addition of biotin increased cGMP content 3-fold within 1 h and induced a 4-fold increase in the activity of glucokinase (23). Biotin has been reported to regulate the glucokinase gene at the transcriptional stage in starved rats (24).

In a study of glucose transporters in diabetes, a dramatic decrease (tenfold) was observed in the steady-state level of GLUT-4 messenger RNA in adipose tissue from rats deficient in insulin using STZ (25). In fact, an insulin-sensitive glucose transporter is the primary form of carrier responsible for insulin-induced glucose uptake in insulin-responsive tissues (26–28). On the basis of this study, showing an improvement in glucose metabolism without enhancing insulin secretion, it is postulated that a short duration of biotin treatment may effect an improvement in disorders occurring in hepatocytes in diabetes mellitus that involve glucokinase or glucose transporters. The next step will be to investigate the effect of biotin on the glucose transporters.

Finally, we should point out that the precise relationship between the extent of the functional disorder and the physiological role of biotin is, as yet, unclear in STZ-rats used in this study.

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