A High Biotin Diet Improves the Impaired Glucose Tolerance of Long-Term Spontaneously Hyperglycemic Rats with Non-Insulin-Dependent Diabetes Mellitus

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Summary The Otsuka Long-Evans Tokushima Fatty (OLETF) rat, serving as a spontaneously diabetic model with non-insulin-dependent diabetes mellitus (NIDDM), exhibits impaired glucose tolerance (IGT) at about 16 weeks of age. In this study, we investigated whether or not biotin, a water-soluble vitamin, improved the IGT of OLETF rats. To this end, we administered diets containing one of three levels of biotin, a high-biotin diet (BH), a normal-biotin diet (BN) and a basal-biotin diet (BB), to OLETF rats up to 24 weeks of age. An oral glucose tolerance test (OGTT) was performed four times between 13 and 22 weeks of age. The administration of a BH corrected the IGT of OLETF rats. Upon further investigation, we found that insulin secretion in the OLETF-BH rats was decreased to a significant extent, signaling that the hyperinsulinemia typical to the OLETF-BH rats had clearly improved. Body weights were significantly lower in the OLETF-BH group than in the other OLETF groups, even though the OLETF-BH rats showed a significantly higher average daily food intake. The body weight gain of the OLETF-BH rats followed the same tendency as the control-LETO (Long Evans Tokushima Otsuka) rats (LETO-BB and LETO-BN). These results demonstrate that a high-level biotin diet can improve the glucose handicap in NIDDM rats.

Key Words biotin, egg white, oral glucose tolerance test, NIDDM (non-insulin-dependent diabetes mellitus), insulin secretion

It has been found that biotin deficiency results in an impairment of glucose utilization (1–5). Moreover, the reduced oral glucose tolerance and insulin response to oral glucose loads seen in mice with non-insulin-dependent diabetes

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("diabetic KK mice") can be improved by feeding with a diet containing 2-4 mg of biotin per kg for eight weeks, as shown by Raddi et al. (6). Furthermore, they demonstrated that a pharmacologic dose of biotin (16 mg/day for one week) lowered the plasma glucose concentration in insulin-dependent diabetic patients during insulin withdrawal. In clinical studies, we found (i) that the serum biotin levels in psoriasis vulgaris and pustulosis palmaris et plantaris (ppp) patients are significantly lower than in normal subjects and (ii) that the administration of biotin (9 mg per day) to those patients can improve a variety of clinical symptoms as well as associated metabolic abnormalities (7). Subsequently, we evaluated the therapeutic effectiveness of biotin in patients with non-insulin-dependent diabetes mellitus (NIDDM) and sternocostoclavicular hyperostosis (8, 9).

In a long-term experiment using Osteogenic Disorder Shionogi (ODS) rats, which have a hereditary defect in their ability to synthesize ascorbic acid and which were fed a biotin-deficient diet containing dried egg white and sufficient ascorbic acid, we observed a decrease in plasma insulin at an early stage of biotin deficiency (10). The insulin secretion in response to an oral glucose load (1.8 g glucose solution per kg body weight) in biotin-deficient Wistar male rats was approximately one-sixth less, in concentration terms, than that of pair-fed control rats (11). However, the insulin content of the pancreas in the same biotin-deficient rats was no lower than that of the control rats. The reduction in insulin secretion of these biotin-deficient rats was significantly improved by the simultaneous administration of biotin (1 mg per kg body weight) with the glucose solution (11). Despite the accumulation of such reports, the metabolic mechanism underlying these effects remains unclear.

In this study, we used a new strain of rat (OLETF) with spontaneous persistent hyperglycemia in the absence of insulin therapy. In the chronic course of their diabetes mellitus, OLETF rats resemble humans with NIDDM (12). This study aimed to establish (i) whether a relationship exists between biotin concentration and the extent of glucose tolerance, and (ii) whether biotin deficiency might be one of the factors contributing to impaired glucose tolerance in non-insulin-dependent diabetic rats.

MATERIALS AND METHODS

Animals, diets and experimental design. Male OLETF and LETO rats were obtained from Tokushima Research Laboratories, Otsuka Pharmaceutical Co. Ltd. (Tokushima, Japan). The characteristic signs and features of the OLETF-strain rat are: 1) polyuria, polydipsia and mild obesity; 2) late onset hyperglycemia; 3) the chronic course of the disease; 4) inheritance by males; and 5) nervousness. We also used LETO rats as the control animal in this study. The LETO strain was obtained by different original matings from those for OLETF rats. Both strains originated from the same colony of Long-Evans rats. The LETO strain had not shown any signs of diabetes. 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 0800 to 2000. Rats of the OLETF and LETO strains, four weeks old and specifically pathogen-free, weighing approximately 80 and 60 g, respectively, were habituated by feeding a commercially available solid feed (Type F-2, Funabashi Farm Co., Funabashi, Japan) for one week before initiation of the experiments involving purified diets. All animals were kept on the BB diet for eight weeks of the study from the age of five weeks to 13 weeks old. Then, the OLETF rats were given one of the three biotin diets (BB, BN or BH). The LETO rats were divided into two groups which were given either the BB or BN diet during a nine-week experimental period (13 weeks to 22 weeks old). The composition of the basal diet, containing 20% spray-dried egg white as the protein source, was exactly the same as described previously (10), and three experimental diets with different biotin levels were prepared as shown in Table 1. The diet and distilled water were provided ad libitum. The average body weight of each group was determined once every week.

**Oral glucose tolerance test (OGTT) and insulin secretion in response to an oral glucose load.** All rats underwent an OGTT to provide a profile of their original

Table 1. Composition of experimental diet.

| Ingredients                  | Amount (%) |
|------------------------------|------------|
| Sucrose                      | 60         |
| Egg white<sup>1</sup>        | 20         |
| Soybean oil<sup>2</sup>      | 6          |
| Salt mixture<sup>3</sup>     | 6          |
| Cellulose powder<sup>4</sup> | 6          |
| Vitamin mixture<sup>5</sup>  | 2          |

<sup>1</sup> Spray-dried egg white protein from Q.P. Corporation, Japan.

<sup>2</sup> Wako Pure Chemical Co., Ltd., Osaka, Japan.

<sup>3</sup> Oriental’s Salt Mixture, Oriental Yeast Co., Ltd., Japan.

<sup>4</sup> One of three kinds of vitamin mixture according to biotin level: biotin-basal, biotin-normal or biotin-high level diet.

<sup>5</sup> Biotin content of experimental diets.

| Type          | Basal diet (BB) | Normal diet (BN) | High diet (BH) |
|---------------|-----------------|------------------|---------------|
| Biotin (mg/kg diet) | 1.44<sup>a</sup> | 1.84<sup>b</sup> | 7.44<sup>c</sup> |
| (BB)          | (BB + 0.4)      | (BB + 0.4 × 15)  |               |

<sup>a</sup> One kilogram of the biotin-basal diet contained 200 g spray-dried egg white. Two-hundred grams of the egg white contained 100 mg avidin (1.47 μmol), which bonded to 5.89 μmol of biotin (1.44 mg).

<sup>b</sup> 1.44 mg biotin plus normal biotin content (0.4 mg) of ordinary diet.

<sup>c</sup> Biotin high-level diet (BH) contains 15 times more biotin than the normal biotin level.
oral glucose tolerance during the nine-week experimental period. Subsequently, an OGTT was carried out on each rat in the third (1st OGTT), sixth (2nd OGTT) and ninth (3rd OGTT) week of the dietary regimens. An OGTT was performed without anaesthesia and the insulin secretion of each animal was measured four times over the nine-week experimental 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 of 1.8 g glucose per kg body weight via a gastric catheter. The plasma glucose levels were estimated by a standard glucose oxidase method (14) (Glucose C-II test kit, Wako Pure Chemical Co. Ltd., Osaka, Japan). The immunoreactive insulin of the plasma was determined with a Shionogi Insulin RIA kit (Shionogi Co. Ltd., Osaka, Japan) which employs the double-antibody method of radioimmunoassay (15).

The diagnosis of diabetes mellitus involved the classification of the plasma glucose level by an OGTT (16) into one of the following three types:

1) normal pattern:  
   \(<300\,\text{mg/dl (at 30 min)}\) and \(<200\,\text{mg/dl (at 120 min)}\);

2) impaired glucose tolerance (IGT) pattern:  
   either \(>300\,\text{mg/dl (at 30 min)}\) or \(>200\,\text{mg/dl (at 120 min)}\); and

3) diabetes mellitus (DM) pattern:  
   both \(>300\,\text{mg/dl (at 30 min)}\) and \(>200\,\text{mg/dl (at 120 min)}\).

Determination of biotin concentration in the plasma and organ. The plasma biotin concentration was determined microbiologically by a procedure described previously (13). The test organism was Lactobacillus plantarum ATCC 8014. The samples of plasma and organ homogenates were acid-hydrolyzed prior to the assay.

Statistical analysis. All results are expressed as M±SEM. 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 and symptoms of OLETF and LETO rats

The growth curves for OLETF-BB, -BN and -BH rats and for LETO-BB and -BN rats are shown in Fig. 1. At the start of the experiment using 13-week old rats, the average body weight of the OLETF rats was 400 g and the LETO rats about 350 g. All of the groups of OLETF rats grew at the same rate until 16 weeks old (the first three weeks of the dietary regimen). Thereafter, the OLETF-BH rats grew less than the other OLETF rats. After receiving their experimental diet for about 10 weeks, the average body weight of the OLETF-BH rats was significantly less than that of the OLETF-BB rats. Furthermore, the OLETF-BH rats grew at a rate similar to those of the LETO-BB and LETO-BN rats, with the exception of the difference in body weight at the start of the experiment.
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Fig. 1. Effect of dietary biotin content on the body weight of OLETF and LETO rats. Rats were fed one of three experiment diets with different biotin levels. These contained 20% freeze-dried raw egg white as the protein source. In OLETF rats: biotin basal diet group, OLETF-BB (●); biotin normal diet group, OLETF-BN (▲); biotin high diet group, OLETF-BH (■). In LETO rats: biotin basal diet group, LETO-BB (○); and biotin normal diet group, LETO-BN (△). Results are expressed as M±SEM. *p<0.05, **p<0.01. (0 week is equal to 13 weeks old).

Glucose tolerance profile and plasma glucose level of OLETF and LETO rats

Figure 2 depicts the plasma glucose levels in OLETF and LETO rats given 1.8 g of glucose solution per kg body weight by way of an oral tolerance test. The 13-week old OLETF rats had a glucose level similar to the LETO rats at 0 min. Nevertheless, the OLETF rats showed significantly higher plasma glucose levels than LETO rats at 30, 60 and 120 min after glucose loading. The plasma glucose levels of both the groups of 16-week old OLETF-BB and OLETF-BN rats exceeded 300 mg/dl at 30 min, showing that they could be classified as having IGT. This continued until the end of the 22nd week of growth. However, the average plasma glucose levels of the OLETF-BH group of 16-week old rats was a lower value than the other OLETF groups, but it was not

Effect of dietary biotin level on insulin response to an oral glucose load in OLETF and LETO rats

As shown in Fig. 2, before beginning the three kinds of experimental diet (rats at 13 weeks of age), the LETO rats showed significantly higher insulin levels at 30 min after glucose loading. The average insulin level of the OLETF-BH group of 16-week old rats was a lower value than the other OLETF groups, but it was not
Fig. 2. Effect of glucose on the plasma glucose and insulin response in OLETF rats. In OLETF rats: biotin basal diet group, OLETF-BB (●); biotin normal diet group, OLETF-BN (▲); biotin high diet group, OLETF-BH (■). In LETO rats: biotin basal diet group, LETO-BB (○). Results are expressed as M±SEM, *p<0.05, **p<0.01 (n=8) versus control (LETO) rats. There are significant differences among three groups at p<0.05 (between a and b, b and c or a and c).

significantly different. The levels did not become significantly different from each other during the 120 min after glucose loading. However, the insulin response pattern of the OLETF-BH rats at 19 weeks of age began to show a significant downward shift as compared to the pattern for the OLETF-BB and OLETF-BN rats. The difference was even more significant, the insulin level for the OLETF-BH rats at 22 weeks of age at 30, 60 and 120 min were 47, 43 and 37%, respectively, when comparing only the OLETF-BB group (Fig. 2).

Overall, the insulin secretion in response to the oral glucose loading of OLETF-BH rats was approximately 50% less, in concentration terms, than that of OLETF-BB rats. Specifically, the insulin concentration at each of the four time points (0, 30, 60 and 120 min) in the last experimental stage (after nine weeks on an experimental diet) was significantly reduced by the addition of excess biotin into the diet. Biotin did not alter plasma insulin concentrations in the control-LETO rats (LETO-BB and LETO-BN) after glucose loading during the nine-week exper-
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Biotin content of plasma and organ in OLETF and LETO rats

The plasma biotin concentrations determined at various ages (13, 16, 19 and 22 weeks) are shown in Table 2. OLETF rats 13 weeks of age had a significantly higher concentrations of biotin in their plasma than did the LETO rats. However, throughout the nine-week experimental period, the plasma biotin concentrations of OLETF-BH rats did not rise to a higher level than those of the OLETF-BB rats. As shown in Table 3, the concentrations of biotin in the liver and pancreas of rats in the OLETF-BH group were significantly higher than the other groups of OLETF-BB and OLETF-BN. Nevertheless, the LETO rats (LETO-BB and LETO-BN) showed extremely high levels of biotin in the liver and pancreas. Moreover, the average weight of the pancreases of LETO rats was significantly heavier than that of

Table 2. Changes in the plasma biotin concentration of OLETF and LETO rats.

| Group   | Pre          | 3-week       | 6-week       | 9-week       |
|---------|--------------|--------------|--------------|--------------|
| OLETF-BB| 1.79±0.15<sup>a</sup> | 1.63±0.19<sup>a</sup> | 2.85±0.12<sup>a</sup> | 2.62±0.19<sup>a</sup> |
| OLETF-BN| 1.79±0.15<sup>a</sup> | 1.28±0.16<sup>ab</sup> | 3.41±0.17<sup>a</sup> | 2.31±0.11<sup>a</sup> |
| OLETF-BH| 1.79±0.15<sup>a</sup> | 0.96±0.12<sup>abc</sup> | 2.67±0.27<sup>ab</sup> | 1.97±0.11<sup>ab</sup> |
| LETO-BB | 0.92±0.12<sup>b</sup> | 0.70±0.12<sup>bc</sup> | 1.70±0.25<sup>bc</sup> | 1.59±0.14<sup>bc</sup> |
| LETO-BN | 0.92±0.12<sup>b</sup> | 0.53±0.10<sup>c</sup> | 1.44±0.14<sup>c</sup> | 1.41±0.13<sup>c</sup> |

All rats were repeated for subsequently performed oral glucose tolerance tests (OGTT) during the nine-week experimental period. Pre OGTT (13 weeks old); 1st OGTT (16 weeks old); 2nd OGTT (19 weeks old); 3rd OGTT (22 weeks old). Values are M±SEM for n=8. The differences between the five 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).

Table 3. Organ weights and biotin concentration in the liver and pancreas of OLETF and LETO rats.

| Group   | Liver (g/100 BW) | Pancreas (g) | Biotin concentration (ng/tissue) |
|---------|-----------------|--------------|----------------------------------|
|         |                 |              | Liver                             | Pancreas   |
| OLETF-BB| 3.50±0.50       | 1.05±0.03<sup>a</sup> | 110.8±9.1<sup>a</sup> | 21.7±4.3<sup>a</sup> |
| OLETF-BN| 3.11±0.21       | 1.05±0.05<sup>a</sup> | 147.1±8.4<sup>b</sup> | 26.5±1.3<sup>a</sup> |
| OLETF-BH| 3.04±0.25       | 1.05±0.02<sup>a</sup> | 255.4±11.5<sup>a</sup> | 38.6±3.7<sup>b</sup> |
| LETO-BB | 2.56±0.21       | 1.32±0.07<sup>b</sup> | 364.6±20.3<sup>d</sup> | 126.3±7.9<sup>a</sup> |
| LETO-BN | 2.55±0.22       | 1.33±0.06<sup>b</sup> | 367.8±21.6<sup>d</sup> | 127.4±8.1<sup>c</sup> |

Values are M±SEM for n=5 or 6. Differences between five 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).
the OLETF rats, but the average weights of the liver and pancreas were not significantly different among three groups of OLETF rats (Table 3).

DISCUSSION

The effect of dietary biotin intake on plasma glucose concentration and insulin secretion was investigated in OLETF and LETO rats using an oral glucose tolerance test (OGTT). The OLETF-BB rats showed a characteristic pattern of IGT. However, when the diet was supplemented with a high level of biotin, the plasma glucose level in the OLETF-BH rats became within the normal range before the end of the study (at 22 weeks of age); the improvement was increasingly apparent as the experiment progressed. In the case of NIDDM mice (KK mice) with moderate hyperglycemia (glucose levels remaining at 150–250 mg/dl), the high doses of biotin (2 or 4 mg/kg) administered were remarkably potent in lowering the blood glucose concentration, and improved their IGT. The biotin concentration did not increase in the blood of KK mice treated with biotin (6). This result is the same as that for the OLETF rats used in this study. In addition, a high-level biotin diet improved the hyperinsulinemic secretion in OLETF rats (Fig. 2). Nevertheless, the serum immunoreactive insulin levels after glucose loading did not decrease in biotin-treated KK mice.

In our previous study, the reduction of insulin secretion in biotin-deficient rats was significantly improved by the simultaneous administration of biotin and a glucose solution (11). Berdanier and Marshall (17) suggested that biotin may serve to enhance the synthesis or release of insulin in male adult rats. From these previous results, it was believed that biotin may affect the efficiency with which insulin operates in the glucose metabolism, perhaps by some action at the level of insulin secretion. In this experiment, however, the insulin concentration in the plasma of the OLETF-BH rats was rather diminished (Fig. 2). This result indicates that a dietary high biotin intake can improve the metabolism or/and utilization of glucose without the acceleration of insulin secretion from the pancreas.

In this study, the obesity characteristic to the OLETF rat was clearly seen, and the extent of the body weight gain was negatively correlated with the biotin level in the diet. Nevertheless, a subsequent measurement of the food intake of LETO and OLETF rats at 24 weeks of age showed that the OLETF-BH group had a significantly greater food intake than the OLETF-BB rats ($p<0.01$) (Fig. 3). These results demonstrate that the diet requirement of the OLETF-BH rats improved due to the administration of a high level of biotin.

The mechanisms of improving IGT and decreasing body weight gain by biotin administration via a diet are still unclear, but we can speculate as follows: Li Hsieh and Mistry (18) pointed out that the activity of glucokinase is low in diabetic, fasting and biotin-deficient rats, and that de novo synthesis of the enzyme could be induced by insulin and biotin in intact rat liver. Furthermore, biotin can regulate the glucokinase gene at the transcriptional stage in the liver of fasted rat (19). It
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Fig. 3. The average daily food intake of LETO and OLETF rats of 24 weeks old.
The food intake of each group of rats for 11 days from 24 weeks of age are shown as M(SEM). BB: biotin-basal diet; BH: biotin-high level diet. There are significant differences among the three groups at p<0.05 (between a and b, b and c or a and c).

It is conceivable that biotin could activate glucokinase synthesis in the liver; it follows the enhancement of glucose metabolism. The next step will be to investigate the effect of biotin on the activity of glucokinase and the glucose transporter.

In another study, nicotinamide administered to OLETF rats had an effect similar to that of biotin, in which diabetic complications were significantly inhibited. Moreover, pathophysiological changes (hyperplasia and fibrosis of pancreas islets) were restrained until 65 weeks of age, and the regeneration of β-cells was shown (16). It is as yet unclear if the action of DNA was restored by nicotinamide.

The precise relationship between the degree of functional disorder and biotin concentration in the plasma remains to be clarified in the types of rats used in this study (OLETF and LETO).

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