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Biotin enhances glucose-stimulated insulin secretion in the isolated perfused pancreas of the rat

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The effects of biotin on insulin secretion in pair-fed control rats and biotin-deficient rats were investigated using the method of isolated pancreas perfusion. Isolated pancreas perfusion was performed using 20 mM glucose, 10 mM arginine, and 20 mM glucose plus various concentrations of biotin (20 mM glucose + biotin solution) as stimulants of insulin secretion. The insulin response to 20 mM glucose in biotin-deficient rats was approximately 22% of that seen in control rats. The level of the insulin response to 10 mM arginine was also significantly lower in biotin-deficient rats than in control rats. These results indicate that insulin release from the pancreas was disturbed in biotin-deficient rats. The insulin responses to 20 mM glucose + 1 mM biotin in biotin-deficient and control rats increased to 165% and 185%, respectively, of that to 20 mM glucose. These biotin-induced increases in glucose-stimulated insulin release were evident within the first few minutes of the infusion. An enhancement of the arginine-induced insulin response in control rats was not found when arginine and biotin was administered. These results suggest that biotin may play an important role in the mechanism by which glucose stimulates insulin secretion from the β cells of the pancreatic islets. (J. Nutr. Biochem. 10:237–243, 1999)

Keywords: biotin; isolated pancreas perfusion; glucose; arginine; insulin response; biotin-deficient rat

Introduction

The biochemical role of biotin in carbon dioxide (CO_2) fixation is well established, and many metabolic functions have been ascribed to biotin.[1] Several early reports indicated that (1) biotin deficiency may induce an impairment of glucose utilization,[2–5] (2) there are many similarities in terms of carbohydrate metabolism between diabetic and biotin-deficient rats, and (3) the responses of diabetic and biotin-deficient rats to both biotin and insulin treatment are remarkably similar.[2] For example, in biotin-deficient animals, insulin increases liver glucokinase activity and restores liver hexokinase activity,[6] whereas biotin stimulates the activity of the key glycolytic enzymes glucokinase, phosphofructokinase, and pyruvatekinase in the alloxan diabetic rat.[5] In fact, an effect of biotin on glucose utilization in diabetic patients was suggested by Coggeshall et al.,[7] who demonstrated that the fasting blood glucose concentration in patients with insulin-dependent diabetes was decreased by administration of biotin (16 mg/day for 1 week) rather than insulin. Some time ago, we reported that in the early stages of biotin deficiency in rats, the plasma insulin level during an oral glucose tolerance test was lower than that seen in control animals, even though the plasma glucose levels were almost equal in the two groups.[8] Moreover, this reduced insulin secretion in biotin-deficient rats was improved by the simultaneous administration of biotin (1 mg/kg body weight) with the glucose solution.[9] These observations seemed to suggest that biotin may play an important role in the mechanism underlying glucose-induced insulin secretion. However, Reddi et al.[10] reported that, although the decrease in oral glucose tolerance and the insulin resistance to an oral glucose tolerance load in mice with non-insulin-dependent diabetes (diabetic kk mice) were improved by treatment with 2 to 4 mg biotin per kilogram of diet for 8 weeks, they did not observe any

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Table 1 Composition of the biotin-free diet

Constituents (ingredients)	g/kg Diet
Sucrose	600
Egg white	200
Soy bean oil	60
Mineral mixture*	60
Cellulose	60
Vitamin mixture (biotin free)†	20

*The mineral mixture includes the following salts (g/100 g of salt mixture): $CaHPO_4 \cdot 2H_2O$, 14.56; KH_2PO_4, 25.72; NaH_2PO_4, 9.53; NaCl, 4.66; Ca-lactate, 35.09; Fe-citrate, 3.18; $MgSO_4$, 7.17; $ZnCO_3$, 0.11; $MnSO_4 \cdot 4H_2O$, 0.12; $CuSO_4 \cdot 5H_2O$, 0.03; KI, 0.01.
†The vitamin mixture in the diet provided the following vitamins (mg/kg diet): A, 10,000 IU; D_2, 2,000; E, 100; K_3, 104; B_1, 24; B_2, 80; B_6, 16; B_{12}, 0.01; C, 600; folic acid, 4; Ca-pantothenate, 100; nicotinic acid, 120; para-aminobenzoic acid, 100; inositol, 120; cholin chloride, 4,000.

change in the serum immunoreactive insulin levels. Moreover, Wagle[3] reported that administration of equal amounts of anti-insulin serum increased the blood glucose concentration to the same extent in normal and biotin-deficient rats, indicating that the amount of circulating insulin was not appreciably changed in biotin-deficient animals compared with normal animals. To try to clarify the relationship between biotin and insulin secretion, we investigated the direct effect of biotin on the glucose-stimulated insulin response using the perfused isolated pancreas of the rat. This technique precludes indirect factors, such as the metabolic changes associated with biotin deficiency, from influencing the results.

Methods and materials

Animals and diets

Twenty-six male, 3-week-old Wistar-slc rats were purchased from Funabashi Farm Co. Ltd. (Shizuoka, Japan). All rats were made biotin-deficient by the use (as the only protein source) of 20% egg white (Kewpie Co. Ltd., Tokyo, Japan) in a diet rigidly excluding biotin. Egg white contains avidin, a glycoprotein composed of four essentially identical subunits, which combines strongly with biotin in a highly specific way, and prevents the absorption of the biotin made by the intestinal microflora.[11,12] These animals were then divided into two groups of equal size. Twice per week, the rats in one group (the control group) were given supplementary biotin (0.5 mL saline containing 100 μg biotin by intraperitoneal injection), while the others were given a comparable volume of saline without biotin (biotin-deficient group). The biotin-deficient group was fed a biotin-free diet ad libitum. The control group was given the same diet; each control rat was given the average amount of the diet eaten by the biotin-deficient group the day before. The detailed composition of the diet is shown in *Table 1*. During the experimental period, the rats were kept in individual cages with wire mesh floors (to prevent them eating their own droppings) in a temperature-, humidity-, and light-controlled room (24 to 25°C, 50 ± 5%, 12-hour light-dark cycle, respectively). They were given free access to distilled water. Male Wistar-slc 12-week-old rats also were purchased from Funabashi Farm Co. Ltd., and were used for the experiments that did not require the use of biotin-deficient rats (see *Figures 4 and 5* and *Table 4*).

Determination of the biotin content of plasma and pancreas

In week 8 of the experiments on the effects of biotin-deficiency, all rats were fasted for 12 hours. Blood was collected from the tail vein without administering anesthesia. After the overnight fast, one-half of each group of experimental animals was euthanized by withdrawal of blood from the abdominal aorta under diethyl ether anesthesia. The pancreas was removed immediately and homogenized in 0.04 M phosphate buffered saline (pH 7.2). Samples of plasma or pancreas homogenate were acid-hydrolyzed prior to assay. The biotin concentration in the plasma samples and the biotin content of the samples of pancreas were determined microbiologically, as described by Furukawa et al.,[8] using the test organism *Lactobacillus plantarum* (ATCC8014).

In vitro perfusion of the pancreas

Each pancreas, whether isolated from a biotin-deficient or control rat, was perfused using a modification[13,14] of the method of Grodsky and Fanska.[15] In the isolated pancreas preparation, which included an attached segment of duodenum, the splenic veins were ligated, and the left gastric artery and veins were ligated and cut. The baseline perfusate consisted of Krebs-Ringer bicarbonate buffer (pH 7.6) containing 5.6 mM glucose; 5 mM of each of pyruvate, fumarate, and glutamate; 0.25% bovin serum albumin (Fraction V, Sigma, St. Louis, MO USA); and 4.5% Dextran T70 (mean molecular weight 70,000; Pharmacia AB, Uppsala, Sweden). The perfusate was continually oxygenated in an atmosphere of 95% O_2 and 5% CO_2, and maintained at 37°C. After a 20-minute preincubation period, each pancreas was perfused for 10 minutes with the baseline perfusate. This was followed by a 25- to 30-minute test with 20 mM glucose or 10 mM arginine, a 5-minute interval of perfusion with the baseline perfusate, and finally a 25- to 30-minute test with 20 mM glucose + 1 mM biotin or 10 mM arginine + 1 mM biotin. The flow rate was maintained constant throughout at 1.5 mL/min. Fractions were collected in chilled tubes at 2-minute intervals and stored at −20°C until assayed for immunoreactive insulin (IRI).

Determination of the insulin level

The IRI in the pancreatic effluent was determined by means of a Shionogi Insulin RIA kit (Shionogi and Co. Ltd., Osaka, Japan), which employs the double-antibody radioimmunoassay method.[16]

Statistical analysis

The difference in growth patterns between biotin-deficient and control groups was analyzed by means of an analysis of variance for repeated measures. Statistical analysis of the other data was performed by means of a Student's *t*-test, and a *P*-value of less than 0.05 was considered significant.

Results

Growth profiles and clinical features of biotin-deficient rats

The growth curves for the biotin-deficient and control groups are shown in *Figure 1*. The biotin-deficient group grew significantly less rapidly than the control group from day 42 onward, with the growth of the biotin-deficient group falling progressively further behind that of the control group. The difference in growth patterns between the two groups was significant ($P < 0.001$). By the end of the

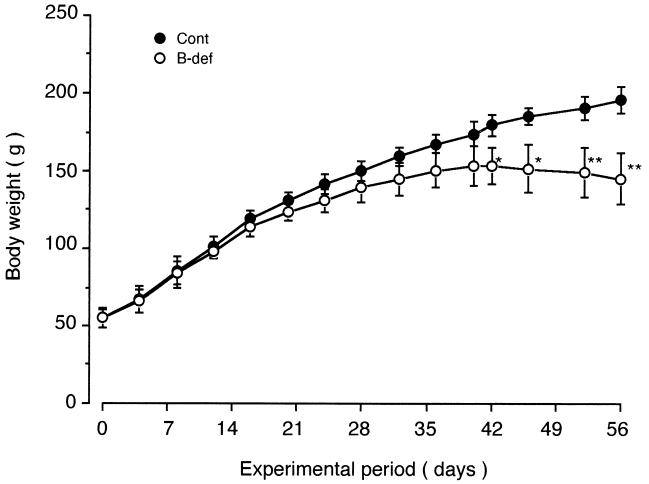

Figure 1 Effect of biotin deficiency on body weight gain in pair-fed control rats (Cont) and biotin-deficient rats (B-def). All rats were fed a biotin-deficient diet containing 20% raw egg white as the only protein source. In addition, control rats were given 100 μg biotin by intraperitoneal injection two times per week. Each point represents the mean ± SD. *$P < 0.01$, **$P < 0.001$ versus control group.

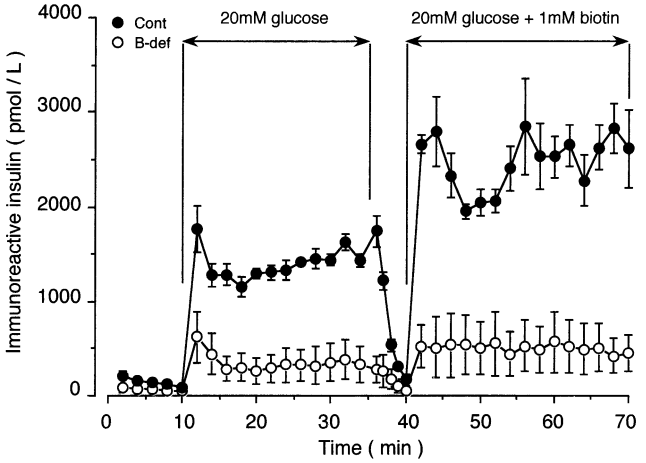

Figure 2 Insulin secretory responses to 20 mM glucose and 20 mM glucose + 1 mM biotin evoked from the isolated perfused pancreas of pair-fed control rats (Cont) and biotin-deficient rats (B-def) in week 8 of the experimental period. Each point represents the mean ± SEM ($n = 4$).

experimental period, the rats of the biotin-deficient group exhibited clinical features of deficiency, including alopecia, dermatitis, and abnormal curvature of the spine, as also reported by Balnave.[17] The body and pancreas weights and the biotin content of the plasma and pancreas in week 8 of the experiment are shown in *Table 2*. In spite of paired feeding, the body and pancreas weights of the biotin-deficient group were both significantly lower than those of the control group ($P < 0.001$ and $P < 0.005$, respectively). The biotin content of both plasma and pancreas were markedly decreased in the biotin-deficient group, to approximately one-fourth of the values found for the control group ($P < 0.001$ in both cases).

In vitro perfusion of the pancreas

The insulin responses to glucose, glucose + biotin, arginine, and arginine + biotin were determined using the technique of isolated pancreas perfusion (*Figure 2*, *Figure 3*, and *Figure 4*). In the control group, the insulin response to 20 mM glucose was maintained at a high level until the end of the stimulation. Furthermore, the integrated insulin response to 20 mM glucose + 1 mM biotin was increased to 185 ± 18% of the response to 20 mM glucose without biotin (*Figure 2* and *Table 3*; $P < 0.025$). However, the response to 10 mM arginine + 1 mM biotin was much the same as that to 10 mM arginine alone (*Figure 4* and *Table 4*; 93 ± 4% of the response to 10 mM arginine, not significant). The insulin response to 20 mM glucose in the biotin-deficient group was markedly lower than that in the control group (*Figure 2*). In fact, this response was significantly lower in the biotin-deficient group than in the control group, at approximately 22% of control (*Table 3*; $P < 0.01$). In the biotin-deficient group, the insulin response

Table 2 Body and pancreas weights, and biotin content of plasma and pancreas in biotin-deficient rats and control rats

	Body weight (g)	Pancreas weight (g)	Biotin content	
			Plasma (ng/mL)	Pancreas (μg/g)
Pair-fed control	196 ± 2	0.99 ± 0.04	31.7 ± 2.5	1.2 ± 0.1
Biotin deficient	153 ± 3[a]	0.76 ± 0.01[b]	8.7 ± 1.1[a]	0.3 ± 0.1[a]

In week 8 of the experimental period, all rats were weighed and then fasted for 12 hours. A blood sample was taken from the tail vein into a heparinized capillary tube for the measurement of plasma biotin content. The pancreas was immediately excised under ethyl ether anesthesia and homogenized in 0.04 M phosphate buffered saline (pH 7.2). The plasma and pancreas biotin contents were determined microbiologically using the test organism (*Lactobacillus plantarum* (ATCC8014). Values are the mean ± SEM. The differences between the biotin-deficient and control rats were analyzed by means of an unpaired Student's *t*-test. [a]$P < 0.001$; [b]$P < 0.005$ vs. control group.

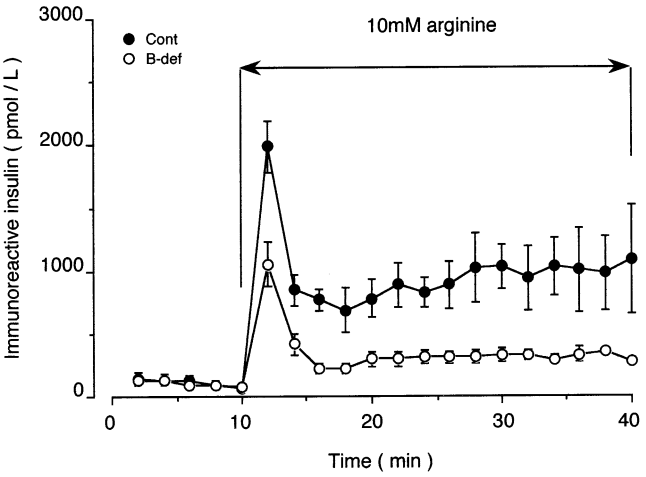

Figure 3 Insulin secretory responses to 10 mM arginine evoked from the isolated perfused pancreas of pair-fed control rats (Cont) and biotin-deficient rats (B-def) in week 8 of the experimental period. Each point represents the mean ± SEM ($n = 4$).

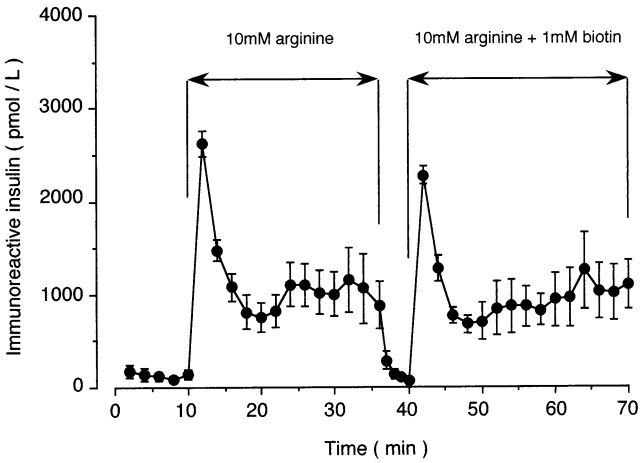

Figure 4 Insulin secretory responses to 10 mM arginine and 10 mM arginine + 1 mM biotin evoked from the isolated perfused pancreas of control rats. Each point represents the mean ± SEM ($n = 4$).

to 10 mM arginine was impaired in much the same way as that to 20 mM glucose (compare *Figure 3* with *Figure 2*; *Table 3*), and the arginine-induced insulin response was significantly lower than that in the control group, at approximately 29% of control (*Table 3*; $P < 0.01$). In the biotin-deficient group, the insulin response to 20 mM glucose + 1 mM biotin was 165 ± 2% of the response to 20 mM glucose without biotin (*Table 3*). This enhancement was observed in all rats, although in the biotin-deficient group the difference did not reach significance because of the large individual variation in the response to 20 mM glucose. The stimulatory effect of biotin on the insulin response to glucose was evident even in the first 10 minutes or so of the period of perfusion both in the control group and in the biotin-deficient group (*Figure 2*).

Relationship between glucose-stimulated insulin secretion and biotin concentration

Figure 5 shows the relationship between the biotin concentration in the perfusate and the insulin responses to glucose + biotin (expressed relative to the response to glucose alone) in control rats. A highly significant correlation ($y = 70.740 + 26.312 * \log(x)$; $r = 0.744$; $P < 0.01$) was noted between the biotin concentration and its effect on glucose-stimulated insulin secretion.

Table 4 Insulin secretion during perfusion of the isolated pancreas with 5.6 mM glucose, 10 mM arginine, or 10 mM arginine plus 1 mM biotin in control rats

	Immunoreactive insulin (pmol/L)
5.6 mM glucose	140 ± 66
10 mM arginine	1003 ± 197
10 mM arginine + 1 mM biotin	930 ± 173
relative insulin release (%)	93 ± 4

Values are means ± SEM and represent the mean integrated insulin response. Relative insulin release was calculated by dividing the integrated insulin secretory response to 10 mM arginine plus 1 mM biotin by that to 10 mM arginine, and expressing the result as a percentage. The difference between responses to 10 mM arginine and 10 mM arginine plus 1 mM biotin was analyzed by a paired Student's *t*-test. A significant difference was not detected.

Discussion

We previously reported that the plasma insulin level in biotin-deficient rats during an oral glucose tolerance test was lower than that in the controls, but was improved when biotin was administered with the glucose solution. This result suggested that it is possible for biotin to influence the secretion of insulin from the pancreas.[9] However, the metabolic mechanism underlying these effects remained unclear. The purpose of the present study was to clarify the relationship between biotin and insulin release using the perfused isolated pancreas. Unexpectedly, in our biotin-sufficient control group, the insulin response to glucose + biotin was significantly greater than the response to glucose alone, just as it was in the biotin-deficient group (*Figure 2*). Furthermore, the increase in the insulin response was

Table 3 Insulin secretion during perfusion of the isolated pancreas with 5.6 mM glucose, 20 mM glucose, 20 mM glucose plus 1 mM biotin, or 10 mM arginine in biotin-deficient and control rats

		Immunoreactive insulin (pmol/L)			
	n	5.6 mM glucose	20 mM glucose	20 mM glucose + 1 mM biotin	Relative insulin release (%)
Pair-fed control	4	164 ± 35	1262 ± 53	2312 ± 228[b]	185 ± 18
Biotin-deficient	4	61 ± 41	273 ± 143[a]	447 ± 220[a]	165 ± 2
		5.6 mM glucose	10 mM arginine		
Pair-fed control	4	114 ± 29	870 ± 146		
Biotin-deficient	4	104 ± 20	250 ± 37[a]		

Values are the mean ± SEM and represent the mean integrated insulin response. The insulin responses to 20 mM glucose, 20 mM glucose + 1 mM biotin, and 10 mM arginine were calculated by subtracting the baseline level (5.6 mM glucose) from the absolute level achieved with each stimulant. Relative insulin release was calculated by dividing the integrated insulin secretory response to 20 mM glucose + 1 mM biotin by that to 20 mM glucose, and expressing the result as a percentage. The differences between the biotin-deficient and control rats were assessed by an unpaired Student's *t*-test. [a]$P < 0.01$ vs. the control rats. The difference between responses to 20 mM glucose and 20 mM glucose + 1 mM biotin was analyzed by a paired Student's *t*-test. [b]$P < 0.025$ vs. 20 mM glucose.

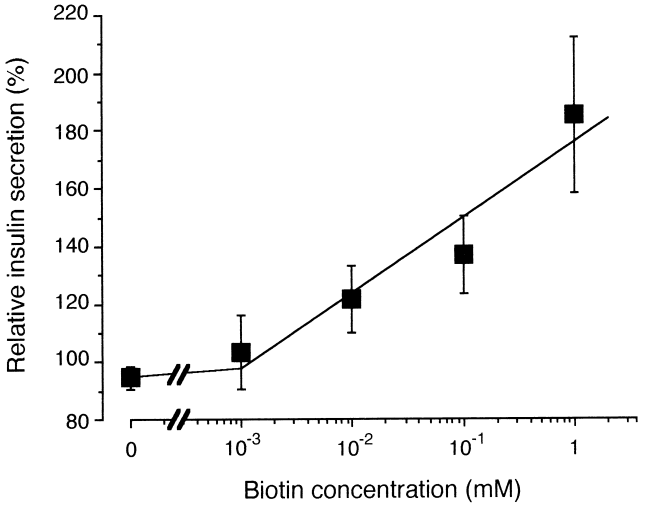

Figure 5 Correlation between biotin concentration in the perfusate and its effect on the insulin secretion from the isolated perfused pancreas of control rats. Relative insulin secretion was calculated by dividing the integrated insulin secretory response to 20 mM glucose plus a given concentration of biotin by that to 20 mM glucose, and expressing the result as a percentage. The integrated insulin secretory response for each stimulus was calculated by subtracting the baseline level (5.6 mM glucose) from the absolute insulin level. Each point represents the mean ± SEM ($y = 70.740 + 26.312 * \log(x)$; $r = 0.744$; $P < 0.01$, $n = 4$).

greater in the control group than in the biotin-deficient group (*Table 3*). Moreover, the relationship between the biotin concentration in the stimulus solution and its effect on the insulin response to glucose showed a highly significant correlation ($y = 70.740 + 26.312 * \log(x)$; $r = 0.744$; $P < 0.01$; *Figure 5*). To judge from the data presented here, it would appear that biotin has an effect that reinforces the glucose-induced insulin response.

Glucose, the major insulin secretagogue, stimulates insulin release following its metabolism via glycolysis and the Krebs cycle. This effect is thought to be mediated by increased production of adenosine triphosphate (ATP), which results in an increase in the cytosolic ATP/adenosine diphosphate (ADP) ratio.[18] The activity of hepatic glucokinase, one of the key glycolytic enzymes, is low in diabetic, fasting, and biotin-deficient rats, and the enzyme's activity is regulated through control of enzyme synthesis at the levels of transcription and translation.[19] Further, several investigators have reported that biotin increases the activity of hepatic glucokinase by a regulating action on the glucokinase gene at the transcriptional stage.[19–22] Matschinsky[23] pointed out that activation of glucokinase in pancreatic β cells may be an important key reaction for glucose-induced insulin secretion. In view of these reports, it seems likely that the influence of glucokinase over the secretion of insulin from pancreatic β cells can be increased by biotin by an action at the transcriptional level. However, the increase in insulin secretion seen in this study was evident within the first 10 minutes or so of the perfusion with biotin-containing solution. Thus, the effect reported here is unlikely to have been mediated via the above-mentioned hypothetical mechanism.

Biotin is a cofactor for the gluconeogenic enzyme pyruvate carboxylase. The pyruvate carboxylase present in islets is not employed for gluconeogenesis because pancreatic islets contain neither the mRNA[24] nor the enzyme activity[25] of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase, which, along with pyruvate carboxylase, catalyzes the conversion of pyruvate to phosphoenolpyruvate. MacDonald has reported that the role of the pyruvate carboxylase present in islets is to participate in a novel supplementary reaction involving four-carbon dicarboxylic acids in the Krebs cycle,[26,27] and that carboxylation of glucose-derived pyruvate is important for glucose-induced insulin secretion.[28] If this is so, it is conceivable that the reinforcement of glucose-induced insulin secretion by biotin could be, at least in part, due to its activation of pyruvate carboxylase, thus catalyzing the entrance of glucose-derived pyruvate into the Krebs cycle, resulting in an increased production of ATP.

Arginine, like glucose, is a well-known secretagogue for the stimulation of insulin secretion from the β cells of the pancreatic islets.[29] In the present study, there was no difference in the insulin response seen in control rats whether L-arginine + biotin or L-arginine alone was used (*Figure 4*). Recently, several mechanisms for arginine-induced insulin secretion have been reported.[30–35] However, there are considerable inconsistencies in the literature as to whether arginine-induced insulin secretion is mediated by nitric oxide production. Weinhaus et al.[36] reported that arginine analogues, which either do not produce nitric oxide or are not metabolizable, caused an insulin release and an increase in the cytosolic calcium concentration ($[Ca^{2+}]_i$) of similar magnitude to those induced by L-arginine. They suggested that the arginine-induced increase in $[Ca^{2+}]_i$ occurs as a result of membrane depolarization caused by cationic amino acids, although the metabolism of arginine itself also may partially contribute to the response. An increase in $[Ca^{2+}]_i$ following the opening of the voltage-sensitive Ca^{2+} channels, which is caused by plasma membrane depolarization, is a feature shared by the pathways mediating the arginine- and glucose-induced insulin secretions.[36–43] This being so, our results seem to suggest that biotin has no significant effect on the distal portion of the signaling pathways involved in insulin secretion. Mobilization of Ca^{2+} from the intracellular stores in the endoplasmic reticulum (ER) is also important in glucose-induced insulin secretion.[44] This event distinguishes glucose- and L-arginine-induced insulin secretion, because the arginine-induced increase in $[Ca^{2+}]_i$ is dependent only upon the influx of extracellular Ca^{2+}.[36] Takasawa et al.[45] suggested that in islet microsomes, cyclic ADP-ribose (cADP-ribose), a metabolite of NAD^+, is the mediator of calcium release, and that it may be generated in islets by glucose stimulation, thus serving as the second messenger for calcium mobilization from the ER. However, at present, it is not possible to state whether biotin enhances the generation of cADP-ribose and/or calcium mobilization from the ER by cADP-ribose. This remains to be examined in future studies.

Leahy et al.,[46] Giroix et al.,[47] and Weir et al.[48] have reported that, in Streptozotocin (STZ)-induced non-insulin dependent diabetes mellitus rats, the insulin response to glucose is markedly impaired, whereas the response to

arginine is almost intact. In contrast, in the present study the insulin responses during perfusion with 20 mM glucose or 10 mM arginine in our severely biotin-deficient group were both significantly weaker than in the control group, at only 22% or 29% of control, respectively (*Table 3*). We observed that the weight of the pancreas was significantly lower in the biotin-deficient group than in the control group (*Table 2*). These results seem to suggest that severe biotin deficiency causes a malfunction in the pancreas that leads to a loss of organ weight and has an adverse effect on the mechanism underlying insulin secretion. However, Dakshinamurti et al.[5] reported nearly 30 years ago that biotin deficiency causes no changes in the histology of the β cells of the islets of Langerhans. Detailed histologic studies in biotin-deficient rats will clearly be required before we can fully understand the true physiologic role of biotin.

In conclusion, the present study has demonstrated that the pancreas of the biotin-deficient rat has an impaired insulin response to both glucose and arginine, and that in biotin-sufficient rats the insulin response to glucose stimulation, but not that to arginine, is enhanced by biotin. This suggests that biotin may play a pivotal role in the mechanism underlying glucose-induced insulin secretion, which includes a glucose recognition process. Further studies are required to elucidate exactly where in this mechanism biotin exerts its influence.

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