

Effects of biotin on pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and markers for glucose and lipid homeostasis in type 2 diabetic patients and nondiabetic subjects^{1–3}

Armida Báez-Saldaña, Iván Zendejas-Ruiz, Cristina Revilla-Monsalve, Sergio Islas-Andrade, Araceli Cárdenas, Alberto Rojas-Ochoa, Alonso Vilches, and Cristina Fernandez-Mejia

ABSTRACT

Background: Several studies have shown that biotin affects glucose homeostasis. Serum biotin concentrations are lower in subjects with type 2 diabetes than in control subjects. Lymphocyte propionyl-CoA carboxylase (PCC; EC 6.4.1.3) activity has proved to be a sensitive indicator of biotin status that is more accurate than is serum biotin concentration.

Objective: We studied the activity of PCC, pyruvate carboxylase (PC; EC 6.4.1.1), and acetyl-CoA carboxylase (ACC; EC 6.4.1.2) in type 2 diabetic and nondiabetic subjects. The effect of biotin administration (6.14 $\mu\text{mol/d}$) on the activity of these enzymes and on several plasma metabolites was also studied.

Design: We compared the activities of carboxylases in circulating lymphocytes from patients with type 2 diabetes ($n = 24$) with those in circulating lymphocytes from nondiabetic subjects ($n = 30$). We also assessed the effect of biotin administration for 14 and 28 d on the activity of these enzymes and on the concentrations of several metabolites (type 2 diabetic patients, $n = 10$; nondiabetic subjects, $n = 7$).

Results: No significant differences in lymphocyte carboxylase activities were found between the type 2 diabetic patients and the nondiabetic subjects. Biotin administration increased the activity of PCC, PC, and ACC in all the subjects. No significant change in glucose, insulin, triacylglycerol, cholesterol, or lactate concentration was observed with the treatment in either the diabetic or the nondiabetic subjects.

Conclusions: The activity of carboxylases does not differ significantly between type 2 diabetic and nondiabetic subjects. Pharmacologic doses of biotin increase lymphocyte PCC, PC, and ACC activities. *Am J Clin Nutr* 2004;79:238–43.

KEY WORDS Biotin, type 2 diabetes, propionyl-CoA carboxylase, pyruvate carboxylase, acetyl-CoA carboxylase

INTRODUCTION

Modification of biotin status is implicated in several physiologic conditions: decreased biotin status is observed in pregnancy (1, 2), in the elderly (3), and in athletes (3). Biotin status has also been found to be altered in diabetic patients. Maebashi et al (4) found that serum biotin concentrations in type 2 diabetic patients were significantly lower than those in control subjects. An inverse correlation between serum biotin and fasting blood glucose concentrations has also been observed (4,

5). Furthermore, biotin deficiency has been linked to impaired oral-glucose-tolerance tests and decreased utilization of glucose in rats (6, 7).

In addition, the diabetic state appears to be ameliorated by biotin treatment. Hyperglycemia reduction was observed in both type 1 and type 2 diabetic subjects treated with biotin (4, 5). In hemodialysis patients, pharmacologic doses of biotin improve their oral-glucose-tolerance tests (8). In genetically diabetic KK mice and in OLETF rats, biotin treatment lowers postprandial glucose concentrations and improves tolerance to glucose (9, 10). Moreover, biotin affects the expression of genes that are critical in glucose metabolism (11–13): biotin increases the expression of hepatic (11) and pancreatic (12) glucokinase (EC 2.7.1.1), an enzyme that plays a key role in glucose homeostasis by regulating insulin secretion in response to glucose in the β cells and uptake of glucose in the liver. In contrast, biotin decreases the expression of phosphoenolpyruvate carboxykinase (EC 4.1.1.32), the activity of which is crucial in the regulation of gluconeogenesis (13).

Lymphocyte propionyl-CoA carboxylase (PCC; EC 6.4.1.3) activity is an early and sensitive indicator of biotin status that has been shown to be more accurate than is plasma biotin concentration (3, 14–16). In the present study, we assessed the activity of PCC in type 2 diabetic patients and in nondiabetic subjects. We also analyzed the activities of pyruvate carboxylase (PC; EC 6.4.1.1) and acetyl-CoA carboxylase (ACC; EC 6.4.1.2), 2 critical enzymes in gluconeogenesis and lipid synthesis, which are metabolic pathways known to be altered in

¹ From the Departamento de Inmunología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City (AB-S); the Unidad de Genética de la Nutrición Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México and the Instituto Nacional de Pediatría, Mexico City (AR-O, AV, and CF-M); the Unidad de Enfermedades Metabólicas, Instituto Mexicano del Seguro Social Siglo XXI, Mexico City (CR-M, SI-A, and AC); and the Departamento de Cirugía, Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico City (IZ-R).

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³ Address reprint requests to C Fernandez-Mejia, Avenida del Iman #1, 4th floor, CP 04530 Mexico City, Mexico. E-mail: crisfern@servidor.unam.mx. Received December 20, 2002.

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diabetes. Moreover, we determined the effect of biotin administration on the activity of these enzymes and on different plasma metabolites and enzymes.

SUBJECTS AND METHODS

Subjects

A total of 39 patients (20 women, 19 men) who had a history of type 2 diabetes mellitus of ≤ 3 y, were aged 33–70 y ($\bar{x} \pm$ SD: 50.4 ± 7.7 y), and had a body mass index (in kg/m^2) of 20.2–31.4 ($\bar{x} \pm$ SD: 28.6 ± 4.9) were studied. At baseline, these subjects had good glycemic control, as reflected by their mean fasting glucose (194.2 ± 67.8 mmol/L) and glycosylated hemoglobin ($9.3 \pm 0.6\%$) concentrations. Diabetic subjects medically treated with glybenclamide were asked to discontinue their medication 1 wk before the baseline study. As a control group, 42 nondiabetic subjects (29 women, 13 men) aged 30–60 y ($\bar{x} \pm$ SD: 46 ± 7.1 y) with a body mass index of 20.0–30.9 ($\bar{x} \pm$ SD: 27.0 ± 3.6) were studied. The subjects had no prior history of renal, hepatic, immunologic, or endocrine disease. Active smokers, patients with a history of alcohol or drug abuse, patients who were obese (body mass index > 32), and patients whose blood pressure was > 160 mm Hg were excluded from the study. The subjects were instructed to consume their ordinary diet and to maintain their usual daily activity level.

The 2 experimental protocols used in this study were reviewed and approved by the Research Committee at the Mexican Institute of Social Security according to the Institute's ethical standards. Informed written consent was obtained from each subject before the start of the study.

Experimental design

Two different protocols were designed. For the first protocol, venous blood samples were drawn from the subjects after they had fasted overnight for 12 h. Plasma and lymphocytes were obtained to analyze metabolites and the enzymatic activity of carboxylases. The second protocol, in which one-half of the subjects in each group participated, was a double-blind, placebo-controlled study. This study consisted of a baseline phase followed by a 28-d treatment period during which the subjects were randomly administered either placebo or 2.05 μmol (5 mg) biotin 3 times/d. The subjects were instructed to take the test products before meals. On days 0, 14, and 28 of treatment, the study subjects fasted overnight and were asked to arrive at the Metabolic Diseases Department at 0700 for blood samples to be drawn. Plasma and lymphocytes were obtained to analyze metabolites and the enzymatic activity of carboxylases.

Blood collection and analytic methods

Blood samples were collected in evacuated tubes (Becton Dickinson, Franklin Lakes, NJ) for analysis of glucose, insulin, triacylglycerol, and cholesterol. Blood samples used for lymphocyte isolation were collected in tubes containing citrate-dextrose-saline (ACD; Becton Dickinson). Plasma glucose, glycosylated hemoglobin, triacylglycerol, and cholesterol concentrations were measured with an automated analyzer (Kodak DT-60C; Kodak, Rochester, NY). Plasma was deproteinized with 6% (wt:vol) perchloric acid for lactate determination, and

TABLE 1

Activities of carboxylases in circulating lymphocytes isolated from type 2 diabetic patients and nondiabetic subjects¹

	ACC	PC	PCC
	<i>nmol CO₂ fixed · min⁻¹ · g protein⁻¹</i>		
Type 2 diabetic patients (<i>n</i> = 24) ²	11.7 ± 1.5	47.8 ± 5.2	557.4 ± 40.7
Nondiabetic subjects (<i>n</i> = 30)	10.7 ± 1.2	43.8 ± 4.0	456.2 ± 27.4

¹ $\bar{x} \pm$ SEM. ACC, acetyl-CoA carboxylase; PC, pyruvate carboxylase; PCC, propionyl-CoA carboxylase. There were no significant differences between the groups (Student's *t* test).

² *n* = 23 for ACC.

lactate concentrations were measured with a commercially available kit (Roche, Mannheim, Germany). Plasma insulin concentrations were measured with a commercially available radioimmunoassay kit (ICN, Costa Mesa, CA).

Lymphocytes were isolated by using a density gradient (Linfograd; Microlab, Mexico City) as previously reported (15). Lymphocyte pellets were resuspended in 1 mL lysis buffer (50 mmol tricine/L, 0.025 mmol EDTA/L, pH 8.0) and disrupted with an ultrasonic homogenizer (4710 series; Cole Palmer Instrument Co, Chicago) at 24 W. Lymphocyte PCC and PC activity was determined by using a radioenzymatic assay as previously described (15, 17). ACC activity was determined by using a similar radioenzymatic procedure with a reaction mixture consisting of the following: 200 mmol tricine/L (pH 8.0), 5 mmol reduced glutathione/L, 1 mmol EDTA/L, 12 mmol magnesium acetate/L, 6.6 mmol ATP/L, 100 mmol potassium acetate/L, 20 mmol $\text{NaH}^{14}\text{CO}_3/\text{L}$ (0.52 GBq/mmol), 0.9 mmol ACC/L, and 4.5 mmol sodium pyruvate/L.

Statistical analysis

Calculations were performed with the SPSS statistical package (version 11; SPSS Inc, Chicago). Results are expressed as means \pm SEMs. Individual comparisons were evaluated by using Student's paired *t* test. Multiple comparisons were evaluated by using 3-factor repeated-measures analysis of variance with interaction. Bonferroni correction was used for the post hoc detection of significant differences. Correlation was performed by using the Pearson's product-moment test. A *P* value ≤ 0.05 was considered statistically significant.

RESULTS

Carboxylase activities

PCC activity was analyzed in circulating lymphocytes obtained from type 2 diabetic patients and nondiabetic subjects. As shown in **Table 1**, no significant difference in PCC activity was found between the nondiabetic subjects and the diabetic patients. No significant correlation was observed between fasting glucose concentrations and PCC activity in either of the groups (overall *r* = 0.182).

We also analyzed the activities of PC and ACC, 2 key enzymes in gluconeogenesis and lipid synthesis, which are essential metabolic pathways known to be affected in diabetes. No significant differences in the activities of these enzymes were found between the 2 groups (Table 1). No significant correlation between fasting glucose concentrations and the

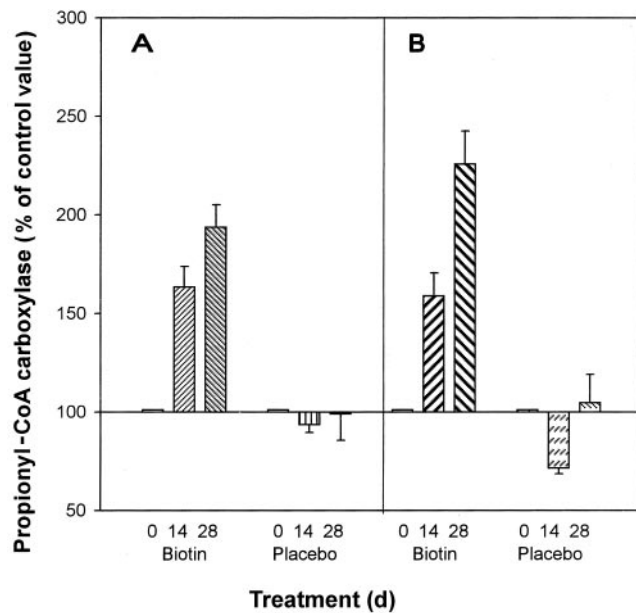


FIGURE 1. Mean (\pm SEM) percentages of control values for lymphocyte propionyl-CoA carboxylase activity in type 2 diabetic patients (A) and nondiabetic subjects (B) after 14 or 28 d of treatment with either biotin (6.14 μ mol/d; $n = 10$ and 7 in the diabetic patients and the nondiabetic subjects, respectively) or placebo ($n = 5$ in each group). Three-factor repeated-measures ANOVA with interaction (diabetic state, treatment, time) was used for statistical analysis. The 3-factor interaction of diabetic state by treatment by time was not significant. The 2-factor interaction of changes over time by treatment (biotin or placebo) with the 2 diabetic states (diabetic and nondiabetic) combined was significant ($P < 0.05$), but the 2-factor interactions of diabetic state by treatment and diabetic state by time were not significant. The main effect of treatment was significant ($P < 0.05$), but the main effect of diabetic state was not significant.

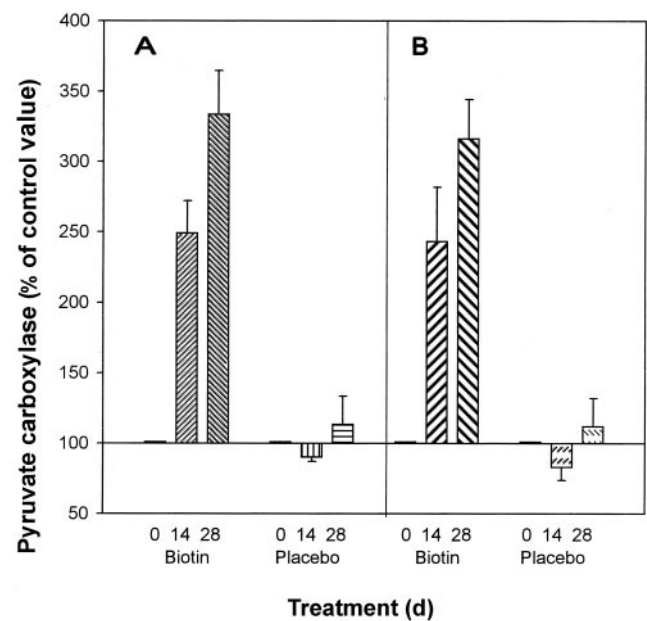


FIGURE 2. Mean (\pm SEM) percentages of control values for lymphocyte pyruvate carboxylase activity in type 2 diabetic patients (A) and nondiabetic subjects (B) after 14 or 28 d of treatment with either biotin (6.14 μ mol/d; $n = 10$ and 7 in the diabetic patients and the nondiabetic subjects, respectively) or placebo ($n = 5$ in each group). Three-factor repeated-measures ANOVA with interaction (diabetic state, treatment, time) was used for statistical analysis. The 3-factor interaction of diabetic state by treatment by time was not significant. The 2-factor interaction of changes over time by treatment (biotin or placebo) with the 2 diabetic states (diabetic and nondiabetic) combined was significant ($P < 0.05$), but the 2-factor interactions of diabetic state by treatment and diabetic state by time were not significant. The main effect of treatment was significant ($P < 0.05$), but the main effect of diabetic state was not significant.

activities of these 2 carboxylases was found in either of the groups (fasting glucose and ACC: overall $r = -0.0128$; fasting glucose and PC: overall $r = -0.0644$).

Effect of biotin administration on carboxylases

We assessed the effect of biotin (6.14 μ mol/d) or placebo administration on PCC activity in lymphocytes from nondiabetic subjects and type 2 diabetic patients. As shown in **Figure 1**, administration of biotin increased PCC activity. Significant increases ($P < 0.05$) at 28 d of biotin treatment were observed in both groups. No significant differences were found between the type 2 diabetic patients (panel A) and the nondiabetic subjects (panel B). Placebo administration did not significantly affect PCC activity in either group.

We also studied the effect of biotin administration on the activity of PC and ACC. As shown in **Figure 2**, a pronounced increase in PC activity was observed with the treatment. Significant increases ($P < 0.05$) at 28 d of biotin treatment were observed in both groups. No significant differences were observed between the diabetic patients (panel A) and the nondiabetic subjects (panel B). Placebo administration did not significantly affect enzyme activity. Biotin treatment also increased ACC activity. Significant increases ($P < 0.05$) of $\approx 90\%$ were observed at 28 d of treatment in both groups (**Figure 3**). No significant effects were seen with placebo administration.

Effect of biotin administration on plasma metabolites

The increases in the activities of lymphocyte ACC and PC, 2 critical enzymes in gluconeogenesis and lipid synthesis, that were produced by the administration of biotin raised the question of whether these increases have direct or indirect effects on different metabolites. As shown in **Table 2**, 28 d of biotin treatment (6.14 μ mol/d) had no significant effects on fasting glucose, insulin, triacylglycerol, cholesterol, and lactate concentrations in either the diabetic patients ($n = 10$) or the nondiabetic subjects ($n = 7$). Placebo administration also did not significantly affect any of the measured metabolites in either the diabetic patients ($n = 5$) or the nondiabetic subjects ($n = 5$). Percentage changes from the subjects' initial values in response to either the biotin treatment or the placebo were not significant for any of the metabolites measured.

DISCUSSION

Several studies have shown that biotin affects glucose homeostasis (6–10). Serum biotin concentrations were found to be lower in type 2 diabetic patients than in control subjects (4). Lymphocyte PCC activity and lymphocyte methylcrotonyl-CoA carboxylase (EC 6.4.1.4) activity (based on 3-hydroisovaleric acid excretion in urine) are currently the most sensitive indicators of biotin deficiency (3, 14–16, 18, 19). In the present study, we assessed PCC activity in the circulating lymphocytes of type 2 diabetic patients and nondiabetic subjects. We found that enzyme

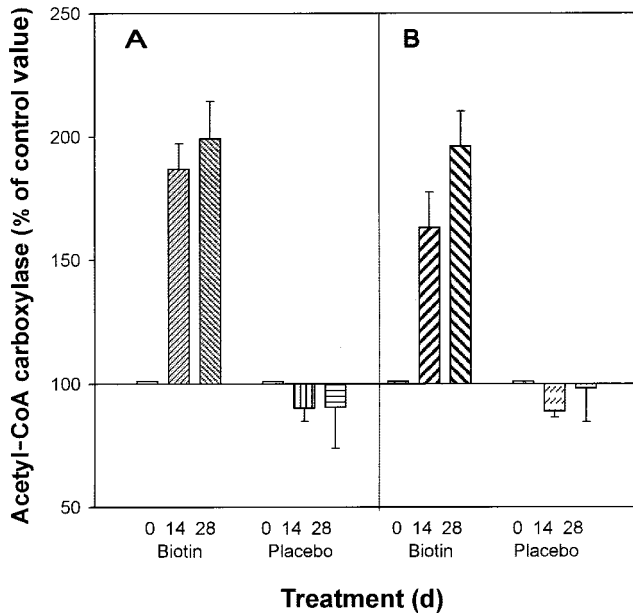


FIGURE 3. Mean (\pm SEM) percentages of control values for lymphocyte acetyl-CoA carboxylase activity in type 2 diabetic patients (A) and nondiabetic subjects (B) after 14 or 28 d of treatment with either biotin (6.14 μ mol/d; $n = 10$ and 7 in the diabetic patients and the nondiabetic subjects, respectively) or placebo ($n = 5$ in each group). Three-factor repeated-measures ANOVA with interaction (diabetic state, treatment, time) was used for statistical analysis. The 3-factor interaction of diabetic state by treatment by time was not significant. The 2-factor interaction of changes over time by treatment (biotin or placebo) with the 2 diabetic states (diabetic and nondiabetic) combined was significant ($P < 0.05$), but the 2-factor interactions of diabetic state by treatment and diabetic state by time were not significant. The main effect of treatment was significant ($P < 0.05$), but the main effect of diabetic state was not significant.

activity did not differ significantly between the groups. Our data also indicate that no correlation exists between fasting glucose concentrations and PCC activity.

In lymphocytes, the activity of PC and ACC did not differ significantly between the diabetic patients and the nondiabetic subjects. In tissues involved in the regulation of glucose homeostasis, PC and ACC activities have been reported to be

altered in the diabetic state (20–24). Because PC and ACC expression is regulated by alternative promoters that are activated under different physiologic conditions and because gene products are heterogeneous and tissue specific (25, 26), it is possible that promoters that are not affected by the diabetic state regulate PC and ACC expression in lymphocytes.

In biotin-deficient patients, as well as in malnourished subjects, biotin administration increases PCC activity (15, 27). In the present study, we showed that the administration of biotin could also increase the activity of this enzyme in subjects without nutritional deficiencies. Our results agree with previous observations by Wolf and Rosenberg (27) and Bartlett et al (28), who found that the administration of biotin increases PCC activity. However, the sample size in both of those studies was small: 2 and 3 patients, respectively. Our work confirms these observations and extends them to a larger number of subjects. In a recent study, Manthey et al (29) found that in the human T cell line Jurkat, pharmacologic doses of biotin (10 nmol/L) increased PCC activity and holo-PCC content; they also found increased contents of holo-PC and holo-methylcrotonyl-CoA carboxylase. In the present study, we found that the activities of PC and ACC can increase as a response to a pharmacologic concentration of biotin. We observed that in human lymphocytes, PC activity was more sensitive to biotin supplementation than were PCC and ACC activities. In contrast, Velázquez et al (15) found that in nutritional deficiencies, PCC is a better indicator of biotin status than is PC.

The increase in PCC activity in response to biotin has been suggested to be useful for detecting patients in whom this enzyme has defects in biotin responsiveness (30). The observation of no significant differences between the diabetic patients and the nondiabetic subjects in the increase in PCC, PC, or AC activity after biotin treatment suggests that the biotin response is not altered in diabetes.

Several studies reported a relation between biotin and lipid metabolism (31–34); indeed, the most prominent features of biotin deficiency are those affecting the skin and the nervous system, 2 tissues in which lipids are important structural components. In the present study, we consistently observed that biotin treatment decreased hypertriglyceridemia, although not

TABLE 2
Effect of biotin or placebo administration on plasma metabolites¹


	Type 2 diabetic patients ($n = 10$)		Nondiabetic subjects ($n = 7$)	
	Baseline	28 d	Baseline	28 d
Biotin				
Glucose (mmol/L) ²	9.30 \pm 0.82	10.53 \pm 1.40	5.75 \pm 0.23	5.92 \pm 0.31
Insulin (nmol/L)	116.2 \pm 22	133.0 \pm 33	105.6 \pm 12	122.4 \pm 11
Triacylglycerol (mmol/L) ²	2.49 \pm 0.39	2.85 \pm 0.32	1.57 \pm 0.3	1.44 \pm 0.2
Cholesterol (mmol/L)	5.05 \pm 0.29	5.16 \pm 0.33	4.68 \pm 0.25	4.63 \pm 0.3
Lactate (mmol/L)	1.88 \pm 0.33	1.27 \pm 0.19	ND	ND
Placebo				
Glucose (mmol/L) ²	11.20 \pm 2.3	9.77 \pm 2.22	5.40 \pm 0.13	5.45 \pm 0.10
Insulin (nmol/L)	178.9 \pm 102	134.8 \pm 41	124.8 \pm 6.8	98.8 \pm 7.64
Triacylglycerol (mmol/L) ²	2.09 \pm 0.36	2.91 \pm 1.49	1.29 \pm 0.22	1.43 \pm 0.32
Cholesterol (mmol/L)	4.92 \pm 0.23	5.5 \pm 0.40	4.83 \pm 0.51	4.97 \pm 0.22
Lactate (mmol/L)	2.05 \pm 0.1	1.88 \pm 0.4	ND	ND

¹ $\bar{x} \pm$ SEM. ND, not determined. None of the 3-factor repeated-measures ANOVAs with interaction (diabetic state, treatment, time) was significant. For lactate, the 2-factor repeated-measures ANOVA with interaction (treatment, time, time \times treatment) was not significant.

² Significant difference between the type 2 diabetic patients and the nondiabetic subjects, $P < 0.05$.

significantly, in subjects whose triacylglycerol concentrations were >2.24 mmol/L, but biotin treatment did not modify hypercholesterolemia. In patients with atherosclerosis and hyperlipidemia, Dukusova and Krivoruchenko (34) found that the administration of biotin ($2.05 \mu\text{mol/d}$ for 4 wk) decreases blood cholesterol concentrations. Furthermore, Marshall et al (33) reported that the largest decrease in blood cholesterol concentrations after biotin administration occurred in patients with exacerbated hyperlipidemia. Hence, the difference in response to biotin between the study by Dukusova and Krivoruchenko (34) and the present study might be related to the different cholesterol concentrations of the patients studied: 7.55 mmol/L in the hypercholesterolemic patients in the study by Dukusova and Krivoruchenko (34) compared with 5.63 ± 0.20 mmol/L in the patients in our study. Moreover, our observation of a decreasing effect of biotin on hypertriglyceridemia in all the subjects whose triacylglycerol concentration was 25% above the normal standard support the concept that biotin is able to decrease exacerbated hyperlipidemia. Whether biotin could be used for the treatment of hyperlipidemia is an issue that deserves further research.

We and others (12, 35) have shown a decrease in glucose-induced insulin secretion in pancreatic islets isolated from biotin-deficient rats. In addition, biotin increases insulin and pancreatic β cell glucokinase expression, as well as glucose-induced insulin secretion, in cultured pancreatic islets isolated from non-biotin-deficient animals (12). In agreement with the results of the study by Maebashi et al (4), the present study found that in vivo pharmacologic doses of biotin do not significantly increase fasting insulin concentrations. Whether biotin treatment in vivo affects glucose-induced insulin secretion, as observed in vitro, is a topic that must be studied further.

The diabetic state has been shown to be ameliorated by biotin treatment (4, 5, 8–10). Maebashi et al (4) found that biotin doses of $3.7 \mu\text{mol/d}$ for 1 mo decreased fasting hyperglycemia $\approx 50\%$ in Japanese diabetic patients. In the present study, however, biotin administration did not significantly decrease fasting glucose concentrations in diabetic patients. Genetic and nutritional differences between Japanese and Mexican diabetic patients may account for these different responses to biotin. A large, placebo-controlled, cross-sectional study using the oral-glucose-tolerance test is currently underway to outline the characteristics of biotin responsiveness. 

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CF-M, AB-S, and IZ-R participated in the study design. AB-S, IZ-R, CR-M, SI-A, AC, AR-O, and AV participated in data collection. AB-S, IZ-R, and CF-M participated in data analysis. AB-S, AV, and CF-M wrote the manuscript. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

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