Bioavailability of biotin given orally to humans in pharmacologic doses[1–3]

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See corresponding editorial on page 352.

ABSTRACT

Background: Patients with carboxylase deficiency are treated with pharmacologic doses of biotin.

Objective: We sought to determine the bioavailability of biotin at pharmacologic doses.

Design: Biotin was administered orally (2.1, 8.2, or 81.9 μmol) or intravenously (18.4 μmol) to 6 healthy adults in a crossover design with ≥2 wk between each biotin administration. Before and after each administration, timed 24-h urine samples were collected. Urinary biotin and biotin metabolites were analyzed by an HPLC avidin-binding assay.

Results: Urinary recoveries of biotin plus metabolites were similar (≈50%) after the 2 largest oral doses and the 1 intravenous dose, suggesting 100% bioavailability of the 2 largest oral doses. For unexplained reasons, the apparent recovery of the smallest oral dose was about twice that of the other doses. For all 4 doses, biotin accounted for >50% of the total of biotin and biotin metabolites in urine. Bisnorbiotin (13–23%), biotin-*d*,*l*-sulfoxide (5–13%), bisnorbiotin methyl ketone (3–9%), and biotin sulfone (1–3%) accounted for the remainder. The percentage excretion of biotin was greater when biotin was administered intravenously and for the largest oral dose than for the 2 smallest oral doses.

Conclusion: Our data provide evidence that oral biotin is completely absorbed even when pharmacologic doses are administered. Biotin metabolites account for a substantial portion of total urinary excretion and must be considered in bioavailability studies. We speculate that renal losses of biotin (as a percentage of the dose administered) are moderately elevated when pharmacologic doses of biotin are administered. *Am J Clin Nutr* 1999;69:504–8.

KEY WORDS Biotin, biotin metabolites, bioavailability, humans, urine, multiple carboxylase deficiency

INTRODUCTION

In mammals, biotin serves as a coenzyme in 4 different carboxylases (1). The hereditary disorder multiple carboxylase deficiency is characterized by reduced carboxylase activity (2, 3). Multiple carboxylase deficiency is caused either by a defect of the enzyme biotin-[propionyl-CoA-carboxylase (ATP hydrolyzing)] ligase (holocarboxylase synthetase; EC 6.3.4.10) (2) or by reduced activity of the enzyme biotinidase (EC 3.5.1.12) (3). Both forms of multiple carboxylase deficiency are treated by lifelong oral administration of biotin at pharmacologic doses.

These therapeutic biotin doses were chosen empirically and range from 20 to 82 μmol (5 to 20 mg) per day, although smaller doses may be sufficient to treat biotinidase deficiencies (3). The choice of this range is reasonable given the lack of biotin toxicity at these high doses, but uncertainties about the bioavailability and the metabolic turnover of biotin may also have contributed to the wide range used to treat carboxylase deficiencies. In the present study, we sought to determine the bioavailability of pharmacologic doses of biotin using a chemically specific assay.

SUBJECTS AND METHODS

Subjects

Six adults (4 women, 2 men) participated in this study. The subjects (5 whites, 1 Asian) were aged 34 ± 9 y ($\bar{x}$ ± SD); their body mass index (in kg/m^2) was 21.7 ± 1.2. The subjects were nonsmokers and healthy on the basis of a medical history and physical examination. None took medication for ≥4 wk before entry into the study protocol or during the study. Pregnant women were excluded from this study by a commercial test (QuPID Pregnancy Tests; Stanbio Laboratory, Inc, San Antonio, TX); pregnancy status was confirmed on the day before each biotin administration. Use of vitamin supplements was discontinued 4 wk before the start of the biotin administration protocol. The subjects were instructed to avoid alcohol during the study. The study was approved by the Human Research Advisory Committee of the University of Arkansas for Medical Sciences, Little Rock. Written, informed consent was obtained from all subjects.

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Biotin preparation

The pharmacy at Arkansas Children's Hospital prepared the solutions for oral and intravenous biotin administration from crystalline *d*-biotin (lot no. JB 173; Spectrum Chemical MFG Corp, St Gardena, CA). The stock solution contained 20.5 mmol (5 g) biotin and 0.3 mol (25.2 g) sodium bicarbonate per liter distilled water (final pH 8.2). The biotin concentration in the stock solution was confirmed by an avidin-binding assay (4), which has an analytic error of ±5% (CV). For enteral biotin administration, appropriate aliquots of the stock solution were given orally as described below. For intravenous biotin administration, 1 mL of the stock solution (20.5 μmol biotin) was diluted with 4 mL 0.9% (wt:vol) saline solution; the solution was sterilized by using a 0.22-μm filter. Sterility was confirmed by culture (Department of Microbiology, Arkansas Children's Hospital). The biotin solution was tested for pyrogens according to the instructions provided by the Associates of Cape Cod for the endpoint method of the Pyrotel-T kit (Falmouth, MA). Endotoxin standards were prepared by dilution from the USP endotoxin standard (US Pharmacopoeia Convention, Inc, Rockville, MD). Endotoxin exposure was less than the endotoxin tolerance limit of substances for intravenous administration (5 endotoxin U/kg). The volume of biotin solution that was injected intravenously was measured by weight difference of the syringe before and after administration. Mean injection volume was 4.5 ± 0.1 mL, which delivered 18.4 ± 0.5 μmol (4.5 ± 0.1 mg) biotin.

Study design

To ensure adequate status for a constellation of micronutrients, a multivitamin and mineral supplement (Theragran-M, lot no. MKD81; Bristol-Myers Squibb, New York) was given orally to all subjects each day during the first week of a 2-wk prephase. The composition of the supplement is given in **Appendix A**. During the second week of the prephase, no vitamin or mineral supplement was provided.

After this prephase, each subject was studied as an outpatient in Arkansas Children's Hospital 4 times by using a randomized, crossover design. Studies were separated by ≥2 wk to allow biotin status to return to normal. Return to normal was documented in "predose" samples as judged by a normal urinary excretion of biotin. After an overnight fast ≥8 h, each subject collected a 24-h urine sample (denoted "predose"). Then, the subject received one of the following biotin doses in random order: 18.5 μmol as an intravenous bolus injection, 2.1 μmol orally, 8.2 μmol orally, or 81.9 μmol orally. For oral biotin administration, aliquots of the biotin stock solution were stirred into a small volume of orange juice and swallowed. The cup was rinsed with some orange juice and the juice was swallowed.

After biotin administration, the subjects collected 24-h urine samples (denoted "postdose"). Subjects were requested to store their collection containers at 4°C during the 24-h collection interval. The urine volumes were measured and aliquots were frozen at −20°C until analyzed.

Immediately after biotin administration, the subjects ate a self-selected breakfast provided by the hospital cafeteria. However, foods rich in biotin (eg, egg yolk and liver) and those containing raw egg white were prohibited.

HPLC avidin-binding assay

Biotin and biotin metabolite concentrations in urine were quantitated by the HPLC avidin-binding assay (5), including quantitation of biotin sulfone and bisnorbiotin methyl ketone, which were recently identified (6). Excretion rates of the loading doses were calculated by multiplying the concentration of biotin and each metabolite by the volume of the 24-h urine sample. To correct for biotin and biotin metabolites that would have appeared in the urine independent of the loading biotin dose (baseline correction), the excretion rates of biotin and metabolites in predose urine samples were subtracted from the excretion rates of biotin and metabolites in postdose urine samples.

Statistics

The significance of differences among treatment groups (biotin doses) were tested by one-way analysis of variance (ANOVA) using SUPERANOVA 1.11 (Abacus Concepts, Inc, Berkeley, CA). Fisher's protected least-significant-difference procedure was used for post hoc testing (7). Differences were considered significant for $P < 0.05$ after Bonferroni adjustment (8).

RESULTS

In the predose urine samples, we detected biotin, bisnorbiotin, bisnorbiotin methyl ketone, biotin-*d,l*-sulfoxide, and biotin sulfone. The total excretion of biotin plus biotin metabolites in the predose urine samples varied from 0.05 to 0.21 μmol/24 h, depending on the subject. These values agreed well with those published previously for normal subjects (5). The maximal variation in excretion of biotin plus metabolites for a given subject among the 4 predose collections was 0.12 μmol/24 h (maximum: 0.186 μmol/24 h; minimum: 0.064 μmol/24 h).

For the 2 largest oral doses (8.2 or 81.9 μmol biotin) and the 1 intravenous dose (18.4 μmol biotin), ≈50% of each dose was recovered in urine within 24 h (**Figure 1**). The urinary recovery was not significantly different among these 3 doses. This observation suggests that the oral doses were ≈100% bioavailable.

In contrast, the recovery of the smallest oral dose as a percentage of the administered dose was about twice as large as that of the intravenous dose and the 2 other oral doses. Specifically, for this 2.1-μmol oral dose, recovery relative to the amount administered was >100% and relative to the recovery of the intravenous dose was ≈266%. Although these observations might suggest that the baseline correction for endogenous biotin excretion did not adequately correct for biotin excretion unrelated to the loading dose, examination of predose excretion and

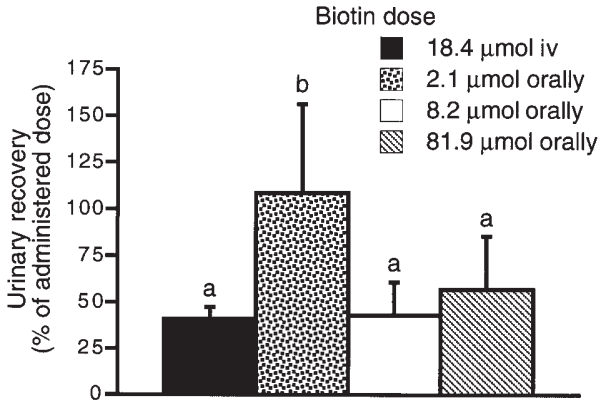

FIGURE 1. Mean (±SD) urinary recovery of biotin plus metabolites within 24 h of biotin administration to 6 adults. Columns with different letters are significantly different, $P < 0.05$. iv, intravenously.

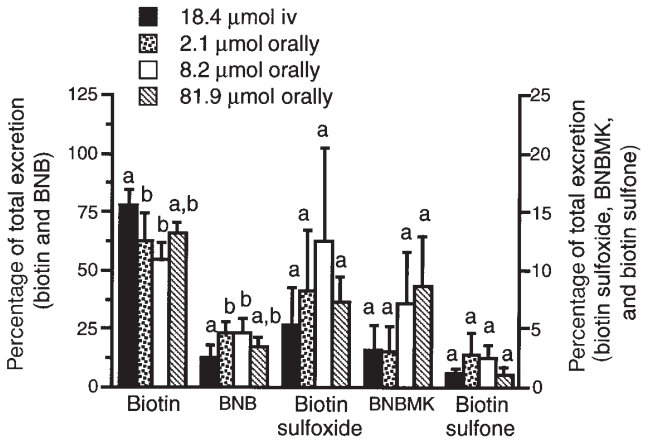

FIGURE 2. Mean (±SD) excretion of biotin and metabolites in urine after administration of pharmacologic doses of biotin to 6 adults. Mean values for different routes of biotin administration of the same biotin compound with different letters are significantly different, $P < 0.05$. Note that excretion rates were amplified by a factor of 5 for biotin sulfoxide, bisnorbiotin methyl ketone, and biotin sulfone (right-hand *y* axis). BNB, bisnorbiotin; BNBMK, bisnorbiotin methyl ketone.

variability (*see* above) revealed that an error in baseline correction could explain only a small part of the theoretically untenable 266% bioavailability.

Biotin accounted for >50% of biotin plus biotin metabolites in the postdose urine samples (**Figure 2**). The percentage biotin was significantly greater for the intravenous dose than for the small and medium oral doses. The percentage biotin was not significantly different among the 3 oral doses.

The quantitative order of biotin metabolites in urine was bisnorbiotin (13–23% of total biotin) >biotin-*d*,*l*-sulfoxide (5–13%) >bisnorbiotin methyl ketone (3–9%) >biotin sulfone (1–3%) (Figure 2). Generally, the percentage excretion of biotin metabolites was smaller for the intravenous biotin dose than for the oral doses. Also, there was a tendency for smaller excretion rates of biotin metabolites for the largest oral dose than for the 2 smaller oral doses.

Bisnorbiotin

The percentage excretion of bisnorbiotin was significantly smaller for the intravenous dose than for the 2 smallest oral doses. There was no significant difference between the intravenous dose and the largest oral dose. The percentage of bisnorbiotin excreted was not significantly different among the 3 oral doses.

Biotin-*d*,*l*-sulfoxide

No significant differences among groups were observed for the percentage excretion of biotin-*d*,*l*-sulfoxide ($P = 0.12$). However, the percentage excretion of biotin-*d*,*l*-sulfoxide showed the same relative relation to dose as did bisnorbiotin. The percentage excretion of biotin-*d*,*l*-sulfoxide was smallest after intravenous biotin administration and was slightly greater after the 2 smaller oral doses than after the largest oral dose.

Bisnorbiotin methyl ketone

No significant differences among groups were observed for the percentage excretion of bisnorbiotin methyl ketone. The differences between the intravenous dose and the medium oral dose ($P = 0.07$) and between the smallest and the largest oral dose ($P = 0.06$) were not quite significant. The percentage excretion of bisnorbiotin methyl ketone was similar after intravenous biotin administration and oral administration of 2.1 μmol biotin. In contrast with the other biotin metabolites, the percentage excretion of bisnorbiotin methyl ketone tended to be greater after the largest oral dose of biotin than after the 2 smaller oral doses.

Biotin sulfone

No significant differences among groups were observed for the percentage excretion of biotin sulfone. The pattern of biotin sulfone excretion was similar to those of bisnorbiotin and biotin-*d*,*l*-sulfoxide. That is, the percentage excretion of biotin sulfone was greatest for the 2 smaller oral doses.

DISCUSSION

Our study provides evidence that biotin supplements are ≈100% bioavailable. Although urinary recovery of the oral doses was only ≈50%, the following finding suggests complete absorption: even when biotin was administered intravenously (ie, when the complete dose enters the systemic circulation), only ≈41% of the dose was recovered in urine. Hence, we did not expect 100% excretion in urine after oral administration of biotin, even with complete absorption.

In the present study, the urinary excretion of biotin metabolites accounted for approximately one-fourth to one-half of the total excretion, ie, biotin plus metabolites. This finding agrees with previous reports (5, 6) and emphasizes the importance of using assays that are chemically specific for biotin and its individual metabolites. Total avidin-binding assays have the disadvantage that biotin compounds are not chromatographically separated before quantitation. The results obtained may be misleading because biotin metabolites bind less efficiently to avidin than does biotin (9, 10). As a result, total avidin-binding assays underestimate the true concentration of biotin plus metabolites (5). In the present study, we used an HPLC avidin-binding assay for urine analysis. In this assay, samples are chromatographed before analysis of the individual HPLC fractions by avidin-binding assay against authentic standards for each metabolite (where possible).

The information published previously regarding the bioavailability of biotin was limited in the following ways. First, biotin doses administered in previous bioavailability studies ranged from 0.3 to 3.7 μmol (75 to 900 μg) (11, 12), ie, the doses were smaller than the doses given to treat carboxylase deficiencies. Second, in previous studies, biotin was analyzed by using microorganisms that do not grow on biotin metabolites (12), or by avidin-binding assays that did not take into account the smaller binding affinities of biotin metabolites for avidin compared with that of biotin (11). In these studies, the urinary excretion of oral doses of biotin were not compared with urinary excretion of similar doses of intravenous biotin. Given these limitations, the findings in these previous studies are consistent with our findings. These previous studies estimated biotin bioavailability at 24–58%.

One may reasonably ask why the urinary recovery of biotin was only one-half of the administered dose. The following findings from our previous studies may explain the metabolic fate of the other half:

1) Our HPLC avidin-binding assay consistently detects avidin-binding substances that are not known and cannot be attributed to any of the possible interfering compounds (5, 6). Likely, these are unknown biotin metabolites with reduced avidin affinity. In the present study, these biotin metabolites were not taken into account.

2) Some biotin metabolites do not bind sufficiently tightly to avidin to be detectable by an HPLC avidin-binding assay. For example, we detected the metabolite tetranorbiotin-*l*-sulfoxide in human urine using thin-layer chromatography and derivatization with *p*-dimethylaminocinnamaldehyde (6). Tetranorbiotin-*l*-sulfoxide is not detected by our HPLC avidin-binding assay.

3) Theoretically, biliary excretion of biotin and metabolites could account for some biotin or biotin metabolite excretion. However, in rats we found that only ≈2% of an intravenous dose of [^{3}H]biotin was excreted into bile as biotin and metabolites (13). Hence, we expected that the biliary excretion of biotin and metabolites is rather small in mammals.

The urinary recovery of biotin after the 2.1-μmol oral dose was 2 times greater than the intravenous dose and the 2 larger oral doses. We cannot explain this observation. However, we speculate that the apparently great recovery of the smallest oral dose may partly be an artifact of biotin intake during the self-chosen diets during the 24 h of the postdose urine collection. The relative contribution of dietary biotin intake to total biotin intake (diet plus test dose) is clearly greater for the smallest oral dose than for the 2 largest oral doses or the intravenous dose. Hence, a relatively large dietary biotin intake on the day of administration of the smallest oral dose would result in greater urinary excretion of biotin and make our baseline correction inadequate. Nevertheless, the baseline excretion on the day of administration of the smallest dose would have had to be ≈1 μmol/24 h (about 10 times measured predose excretion) to provide for a 50% recovery and a 100% calculated bioavailability. Such a large baseline excretion is unlikely, based on the variation measured for predose urine samples. Thus, great baseline excretion can explain only a small portion of the 100% recovery that was measured for the smallest oral dose.

Indeed, on the basis of the data presented here, we cannot exclude the possibility that the bioavailability of the smallest oral dose was truly greater than the bioavailability of the 2 larger oral doses. For the 2 larger doses, the bioavailability might have been overestimated because of unknown factors.

The percentage excretion of intact biotin into urine was greater for intravenous administration than for oral administration. We speculate that this increased renal loss of intact biotin is likely due to saturation of the transporter for renal reabsorption of biotin (14). When biotin is infused intravenously, the complete dose is rapidly infused, leading to great peak biotin concentrations in serum and in the glomerular filtrate; for oral biotin, there is a slower influx of biotin from intestine into the blood stream, leading to smaller peak biotin concentrations in the glomerular filtrate. The high concentration of biotin in the glomerular filtrate after intravenous biotin administration may have exceeded the capacity for tubular reabsorption of biotin. Transport studies of biotin into brush border membrane vesicles from human kidney cortex are consistent with this interpretation: the biotin transporter in these brush border membrane vesicles exhibited saturation kinetics; the Michaelis constant was 31 μmol/L (14). In the present study, peak biotin concentrations in urine were ≈13 and ≈0.7 μmol/L after oral administration of the largest and smallest doses of biotin, respectively. Given that these concentrations are averages from 24-h periods after biotin administration, it seems likely that peak biotin concentrations in urine, or more importantly in the glomerular filtrate, were much greater.

In the present study, the percentage excretion of most biotin metabolites tended to be greater for the 2 smaller oral doses than for the intravenous dose or for the largest oral dose. We speculate that the greater percentage of biotin metabolites was the result of a slower rate of excretion of biotin per se after the smaller oral doses, allowing greater tissue uptake and metabolism. The biotin metabolites detected in the present study arise either from β-oxidation (bisnorbiotin and bisnorbiotin methyl ketone) or from sulfur oxidation (biotin-*d,l*-sulfoxide and biotin sulfone) of biotin. Previous studies in adults receiving chronic biotin supplementation (4.9 μmol/d for 14 d) suggested that pathways for biotin catabolism are not easily saturated (15). Hence, saturation of pathways for biotin catabolism is not likely to account for the smaller percentage excretion of biotin metabolites after the largest oral dose and the intravenous dose in the present study.

In summary, our data provide evidence that oral biotin is absorbed nearly completely—even when large doses are given. Administration of biotin in one large dose per day may lead to increased renal losses as biotin per se. Utilization of biotin supplements might be improved if supplements are administered in 2 or 3 smaller doses spread over the day.

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APPENDIX A

Micronutrient contents of supplement provided during the first week of the prephase[1]

Micronutrient	Amount
Biotin	
(nmol)	123
(μg)	30
Vitamin A[2]	
(μmol)	4.6
(IU)	5000
Thiamine	
(μmol)	10
(mg)	3
Riboflavin	
(μmol)	9.0
(mg)	3.4
Vitamin B-6	
(μmol)	15
(mg)	3
Vitamin B-12	
(nmol)	6.6
(μg)	9
Vitamin C	
(mmol)	0.5
(mg)	90
Cholecalciferol	
(nmol)	13
(IU)	400
all-rac-α-Tocopherol acetate	
(μmol)	63
(IU)	30
Niacin	
(μmol)	164
(mg)	20
Folic acid	
(μmol)	0.9
(μg)	400
Pantothenic acid	
(μmol)	46
(mg)	10
Iron	
(μmol)	0.5
(mg)	27
Copper	
(μmol)	31
(mg)	2
Iodine	
(μmol)	1.2
(μg)	150
Zinc	
(mmol)	0.2
(mg)	15
Magnesium	
(mmol)	4.1
(mg)	100
Calcium	
(mmol)	1
(mg)	40
Phosphorus	
(mmol)	1
(mg)	31
Chromium	
(μmol)	0.3
(μg)	15
Molybdenum	
(μmol)	0.2
(μg)	15
Selenium	
(μmol)	0.1
(μg)	10
Manganese	
(mmol)	**0.1**
(mg)	5
Chloride	
(mmol)	0.2
(mg)	7.5
Potassium	
(mmol)	0.2
(mg)	7.5

[1] Theragran-M, lot no. MKD81, Bristol-Myers Squibb, New York; given orally to all subjects each day for 1 wk.

[2] As acetate and β-carotene.

APPENDIX A

(*Continued*)