Effects of Excess Biotin Administration on the Growth and Urinary Excretion of Water-Soluble Vitamins in Young Rats

Hiromi SAWAMURA, Tsutomu FUKUWATARI, and Katsumi SHIBATA

Laboratories of Food Science and Nutrition, Department of Life Style Studies, School of Human Cultures, The University of Shiga Prefecture, Hikone, Shiga 522-8533, Japan

Received June 14, 2007; Accepted August 10, 2007; Online Publication, December 7, 2007 [doi:10.1271/bbb.70381]

To determine the effects of excess biotin administration on growth and water-soluble vitamin metabolism, weaning rats were fed on a 20% casein diet containing 0.00002% biotin, or same diet with 0.04, 0.08, 0.10, 0.20, 0.50, 0.80 or 1.0% added biotin for 28 days. More than 0.08% biotin administration decreased the food intake and body weight gain compared with the levels in control rats. An accumulation of biotin in such tissues as the liver, brain and kidney increased in a dose-dependent manner, and the both bound and free biotin contents in the liver also increased in a dose-dependent manner. An excess administration of biotin did not affect the urinary excretion of other water-soluble vitamins, suggesting no effect on the metabolism of other water-soluble vitamins. The results of the food intake and body weight gain indicated that the lowest observed adverse effect level for young rats was 79.2 mg/kg body weight/day, while the no observed adverse effect level was 38.4 mg/kg/day. These results suggested immediately setting a tolerable upper intake level for biotin.

Key words: no observed adverse effect level (NOAEL); lowest observed adverse effect level (LOAEL); tolerable upper intake level (UL); urine; blood

Biotin is a water-soluble vitamin classified among the B-group of vitamins. In humans, biotin serves as a coenzyme for four carboxylases: pyruvate, acetyl-CoA, propionyl-CoA, and β-methylcrotonyl-CoA. These carboxylases have important roles in fatty acid synthesis, branched-chain amino acid catabolism, odd-chain fatty acid metabolism, and gluconeogenesis. Although dietary biotin deficiency has not been reported in humans, biotin deficiency has caused growth retardation, alopecia, dermatitis and neurological impairment in experimental animals and humans. In addition, biotin is important in the normal reproductive performance and embryonic growth and development of mammals.

Some people have recently been taking 1–10 mg/d of biotin as a medical treatment because biotin has been found to be correlated with certain diseases such as diabetes mellitus, liver, and skin disorders, neurological abnormality, and epilepsy. Biotin is a heterocyclic compound, an imidazolidone ring joined to a tetrahydrothiophene ring. The latter possesses a valeric acid side chain. The structure is unique, and biotin is more toxic than would be expected if a repeated excess dosage is administered. Indeed, single or repeated doses of biotin (total doses of 50 and 100 mg/kg body weight by subcutaneous injection) given to rats resulted in irregularities of the estrus cycle and fetal and placental resorption in pregnant rats, accompanied by decreased uterine weight, reduced glycogen and protein in the uterus, and reduced protein in the liver. However, these studies cannot be regarded as conclusive for human dietary biotin uptake, because of the route of administration. The administration of oral biotin in doses up to 100 mg/day to patients with holocarboxylase synthetase and biotinidase deficiency has not resulted in adverse effects, although the metabolic defect may prevent or mask toxicity. The Japanese Dietary Reference Intake recommendation presents no data on the tolerable upper intake level (UL) for biotin. Biotin toxicity in healthy humans has not been studied, and performing such a study with the risk of an adverse effect would not be permitted. In the present study, we investigated the effects of excess orally administered biotin on the food intake, body weight gain, tissue weight and water-soluble vitamin metabolism in young rats.

Materials and Methods

Chemicals. Vitamin-free milk casein, sucrose, and L-methionine were purchased from Wako Pure Chemical...
Industries (Osaka, Japan). Corn oil was purchased from Ajinomoto (Tokyo, Japan). Gelatinized corn starch, the mineral mixture (AIN-93M)\textsuperscript{16} and vitamin mixture (AIN-93-VX containing 25% choline bitartrate)\textsuperscript{16} were obtained from Oriental Yeast (Tokyo, Japan). Thiamin hydrochloride (C\textsubscript{6}H\textsubscript{5}ClN\textsubscript{5}O\textsubscript{3}H, 337.27), riboflavin (C\textsubscript{17}H\textsubscript{20}N\textsubscript{4}O\textsubscript{6}, 376.37), cyanocobalamin (C\textsubscript{63}H\textsubscript{84}CoN\textsubscript{14}O\textsubscript{17}X\textsubscript{2}, 1355.40), nicotinamide (Nam; C\textsubscript{6}H\textsubscript{12}N\textsubscript{2}O, 122.13), calcium pantothenate (C\textsubscript{15}H\textsubscript{23}N\textsubscript{2}O\textsubscript{7}Ca, 476.54), folic acid (pteroylmonoglutamic acid; C\textsubscript{19}H\textsubscript{20}N\textsubscript{7}O\textsubscript{6}H\textsubscript{5}, 441.40), d(+)-biotin (C\textsubscript{10}H\textsubscript{16}N\textsubscript{2}O\textsubscript{3}S, 244.31), and L(+)-ascorbic acid (C\textsubscript{6}H\textsubscript{8}O\textsubscript{6}, 176.13) were purchased from Wako Pure Chemical Industries. N\textsuperscript{-}methylnicotinamide (MANA) chloride (C\textsubscript{6}H\textsubscript{4}N\textsubscript{2}O\textsubscript{3}HCl, 159.61) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N\textsuperscript{1}-methyl-2-pyridine-5-carboxamide (2-Py; C\textsubscript{7}H\textsubscript{6}N\textsubscript{2}O\textsubscript{2}, 152.15) and N\textsuperscript{1}-methyl-4-pyridine-3-carboxamide (4-Py; C\textsubscript{7}H\textsubscript{8}N\textsubscript{2}O\textsubscript{2}, 152.15) were synthesized by the methods of Pullman and Colowick\textsuperscript{17} and Shibata \textit{et al.}\textsuperscript{18} respectively. All other chemicals used were of the highest purity available from commercial sources.

\textbf{Animals.} The care and treatment of the experimental animals conformed to the University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals.

Male Wistar rats (3 weeks old) were obtained from CLEA Japan (Tokyo, Japan) and placed in individual metabolic cages (CT-10; CLEA). They were divided into eight groups (such group consisting of four rats) and fed \textit{ad libitum} for 28 days: one group with a 20% casein diet (used as a control group containing 0.00002% biotin), and the others with the same diet plus 0.04, 0.08, 0.10, 0.20, 0.50, 0.80 or 1.0% biotin (Table 1).

The room temperature was maintained at around 22 °C and 60% humidity, and a 12-h light (06:00–18:00)/12-h dark (18:00–06:00) cycle was maintained. The body weight and food intake were measured every 2 days at around 10:00. Urine samples (24 h; 10:00–10:00) were collected in amber bottles containing 1 ml of 1 M HCl on the last day of the experiment, and were stored at −20 °C until needed.

The rats were killed by decapitation at around 10:00 on the last day (day 28), after the urine sample had been collected. Serum was collected to measure biotin, and was stored at −20 °C until needed. The liver, spleen, kidney, heart, lung, brain, testis and thigh muscle of each animal were removed, and a portion (about 0.5 g) was immediately treated as described next to measure biotin.

\textbf{Analyses.} Vitamin B\textsubscript{1}: One milliliter of 1 M HCl was added to 9 ml of the 24-h urine sample. The urinary concentration of thiamin was determined by the HPLC post-labeled fluorescence method.\textsuperscript{19}

Vitamin B\textsubscript{2}: One milliliter of 1 M HCl was added to 9 ml of the 24-h urine sample. The urinary concentration of riboflavin was determined by the HPLC method.\textsuperscript{20}

Vitamin B\textsubscript{6}: One milliliter of 1 M HCl was added to 9 ml of the 24-h urine sample. The urinary concentration of the vitamin B\textsubscript{6} catabolite, 4-pyridoxic acid (4-PIC), was determined by the HPLC method.\textsuperscript{21}

Vitamin B\textsubscript{12}: Part of the 24-h urine samples was stored at −20 °C. The urinary vitamin B\textsubscript{12} concentration was assayed by a microbiological method with \textit{Lactobacillus delbrueckii} subsp. \textit{lactis} ATCC 7830.\textsuperscript{22}

Niacin: One milliliter of 1 M HCl was added to 9 ml of the 24-h urine sample. The quantity of Nam, 2-Py and 4-Py in the urine was measured simultaneously by the HPLC method of Shibata \textit{et al.}.\textsuperscript{18} The content of MNA was measured by the method of Shibata.\textsuperscript{23} The sum of Nam, MNA, 2-Py and 4-Py was used to represent the niacin catabolites.

Pantothenic acid: Part of the 24-h urine samples was stored at −20 °C. The content of pantothenic acid in the urine was directly measured by using \textit{Lactobacillus plantarum} ATCC 8014.\textsuperscript{24}

Folates: One milliliter of 1 M ascorbic acid was added to 9 ml of the 24-h urine sample. The urinary concentration of folates was determined by the microbioassay method with \textit{Lactobacillus casei} ATCC 7469.\textsuperscript{25}

Biotin: Part of the 24-h urine samples was stored at −20 °C. The content of biotin in the urine was directly measured by using \textit{Lactobacillus plantarum} ATCC 8014.\textsuperscript{26} To measure the free serum biotin content, 0.05 ml of serum was added to 1 ml of distilled water, and the mixture was heated for 5 min in a water bath at 100 °C. After cooling to room temperature, the solution was centrifuged at 9000 g for 10 min at 4 °C, and the resulting supernatant was used to measure biotin. To
measure the total biotin content in the tissues, a portion (about 0.5 g) of each tissue (liver, spleen, kidney, heart, lung, brain, testis and skeletal muscle) was homogenized with two volumes of 2.25 M H₂SO₄ and then hydrolyzed by autoclaving for 1 h at 121 °C and 2 atm. After cooling, the hydrolysate was centrifuged at 9000 g for 10 min at 4 °C, and the resulting supernatant was used to measure biotin. To measure the free biotin content in the liver, a portion of the liver was homogenized with two volumes of a 0.05 M potassium phosphate buffer (pH 7.0), the homogenate was centrifuged at 9000 g for 10 min at 4 °C, and the resulting supernatant was used to measure biotin.

Statistical analysis. Each value is expressed as the mean ± SEM. The statistical significance was determined by ANOVA, this being followed by Tukey’s multiple-comparison test. P < 0.05 was considered to be statistically significant. Graph Pad Prism4.0 (Graph Pad Software, San Diego, CA, USA) was used for all the analyses.

Results

Effect of excessive biotin administration on the food intake and body weight gain in young rats

The 0.00002% biotin diet was set as the control because the AIN-93 diet recommended by AIN contains 0.00002% biotin.16) The food intake and body weight gain were not significantly different between the 0.04% biotin-added and control groups, whereas the food intake and body weight gain in the group with the >0.08% biotin-added diets were significantly lower than those in the control group (Table 2). Diarrhea was observed in the young rats fed with the >0.50% biotin-added diets. One rat in four died with the 0.80% biotin-added diet, and two in four rats died with the 1.0% biotin-added diet. Therefore, the data for the 1.00% biotin-added diet group are not shown in Table 2.

Effect of excessive biotin administration on the tissue weight of young rats

Table 3 shows the tissue weight of the rats fed on the biotin diets. The brain weight was not significantly different among the seven groups. The weights of other tissues, including the liver, heart, kidney, lung, spleen and testis, showed increasingly lower values in a dose-dependent manner. The tissue weights in the 0.04% biotin-added group were the same as those in the control group, and all tissue weights except the brain in the 0.50% and 0.80%–added groups were lower than those in the control group. The heart and kidney weights in the groups with the >0.08% biotin-added diets were lower than those in the control group, the liver and lung weights in the groups with the >0.10% biotin-added diets were lower, and the tests weights in the group with the >0.20% biotin-added diets were lower those in the control group.

Effect of excessive biotin administration on the biotin concentration in the urine, tissues and blood

The effect of excessive biotin on the concentration of biotin in the urine is shown in Fig. 1A. The urinary excretion of biotin increased with increasing dietary intake of biotin. The urinary biotin excretion rate to biotin intake was 54.1 ± 5.8 in the control group, and 82.7 ± 2.5, 68.9 ± 5.7, 63.3 ± 2.5, 60.0 ± 2.6, 31.4 ± 6.8 and 29.8 ± 6.8% in the 0.04, 0.08, 0.10, 0.20, 0.50 and 0.80% biotin-added groups, respectively. The serum free biotin content also increased with increasing intake of biotin (Fig. 1B).

The liver total, bound and free biotin contents are shown in Fig. 2. These biotin contents in the liver increased in a dose-dependent manner, although the
liver bound biotin contents were at the same level in the 0.04, 0.08 and 0.10% biotin-added groups. Ninety seven percent of the liver total biotin existed as bound biotin in the control group, and 30–50% of total biotin was of the bound type in the 0.04, 0.08, 0.10 and 0.20% biotin added-groups.

The concentrations of biotin in the skeletal muscle, brain, heart, kidney, lung, spleen and testis also increased with increasing intake of biotin (Fig. 3). The levels of biotin in the skeletal muscle, brain, heart, kidney, lung and testis in the 0.04, 0.08, 0.10 and 0.20% biotin added-groups were significantly higher than those in the control groups. The spleen biotin content in the 0.04% biotin-added group was not significantly higher than that in the controls, however, the biotin contents in the 0.08, 0.10 and 0.20% biotin adding groups were significantly higher than that in the control group.

**Effect of excess biotin administration on the urinary excretion of other water-soluble vitamins**

A mega-dose of biotin did not greatly affect the urinary excretion of other water-soluble vitamins (Fig. 4). Only the urinary excretion of folates was significantly increased by feeding a diet containing up to 0.10% biotin. The urinary excretion of ascorbic acid tended to increase with increasing intake of biotin, but a small number of rats in each group failed to show any significant difference.

**Discussion**

We have previously reported that a 0.3% nicotinamide diet and 1.0% calcium pantothenate diet did not show any effect on the growth of young rats. In the present study, an extremely high dose of biotin representing more than a 0.80% in the diet caused death, and more than 0.08% biotin-added diet retarded the growth of young rats. These results suggest that an excess biotin intake might cause some adverse effects on humans, and that setting UL for biotin would be important to prevent such dietary biotin-induced adverse effects. Although no adverse effects of biotin on humans have been reported, two studies have reported that subcutaneously administered biotin (50 and 100 mg/kg) to pregnant rats inhibited fetal and placental growth and resorption of fetuses and placentae. The effects of excess biotin intake on the reproductive organs of male rats were not investigated in the present study, although the testis weights in the young rats fed with the diets containing more than 0.50% biotin were lower than those in the other groups. Whether an oral intake of high biotin by pregnant rats would affect the sex hormones, reproductive organs and fetal growth remains to be elucidated.

For increasing accumulation of biotin in the tissues was observed as the amount of biotin administered was increased. This phenomenon might have been due to too great an amount of biotin than was possible to metabolize and excrete. It is suggested that this accumulation was associated with the retardation of growth. The bound biotin content in the liver increased in the present study in a dose-dependent manner, and biotin quantification after SDS–PAGE separation showed that 40% of the accumulated biotin in the rat liver bound biotin contents were at the same level in the 0.04, 0.08 and 0.10% biotin-added groups. Ninety seven percent of the liver total biotin existed as bound biotin in the control group, and 30–50% of total biotin was of the bound type in the 0.04, 0.08, 0.10 and 0.20% biotin added-groups.

The concentrations of biotin in the skeletal muscle, brain, heart, kidney, lung, spleen and testis also increased with increasing intake of biotin (Fig. 3). The levels of biotin in the skeletal muscle, brain, heart, kidney, lung and testis in the 0.04, 0.08, 0.10 and 0.20% biotin added-groups were significantly higher than those in the control groups. The spleen biotin content in the 0.04% biotin-added group was not significantly higher than that in the controls, however, the biotin contents in the 0.08, 0.10 and 0.20% biotin adding groups were significantly higher than that in the control group.

**Effect of excess biotin administration on the urinary excretion of other water-soluble vitamins**

A mega-dose of biotin did not greatly affect the urinary excretion of other water-soluble vitamins (Fig. 4). Only the urinary excretion of folates was significantly increased by feeding a diet containing up to 0.10% biotin. The urinary excretion of ascorbic acid tended to increase with increasing intake of biotin, but a small number of rats in each group failed to show any significant difference.

**Discussion**

We have previously reported that a 0.3% nicotinamide diet and 1.0% calcium pantothenate diet did not show any effect on the growth of young rats. In the present study, an extremely high dose of biotin representing more than a 0.80% in the diet caused death, and more than 0.08% biotin-added diet retarded the growth of young rats. These results suggest that an excess biotin intake might cause some adverse effects on humans, and that setting UL for biotin would be important to prevent such dietary biotin-induced adverse effects. Although no adverse effects of biotin on humans have been reported, two studies have reported that subcutaneously administered biotin (50 and 100 mg/kg) to pregnant rats inhibited fetal and placental growth and resorption of fetuses and placentae. The effects of excess biotin intake on the reproductive organs of male rats were not investigated in the present study, although the testis weights in the young rats fed with the diets containing more than 0.50% biotin were lower than those in the other groups. Whether an oral intake of high biotin by pregnant rats would affect the sex hormones, reproductive organs and fetal growth remains to be elucidated.

For increasing accumulation of biotin in the tissues was observed as the amount of biotin administered was increased. This phenomenon might have been due to too great an amount of biotin than was possible to metabolize and excrete. It is suggested that this accumulation was associated with the retardation of growth. The bound biotin content in the liver increased in the present study in a dose-dependent manner, and biotin quantification after SDS–PAGE separation showed that 40% of the accumulated biotin in the rat
liver that have been overdosed with biotin was bound to protein (data not shown). Hymes et al. have proposed a reaction mechanism by which the enzyme, biotinidase (EC 3.5.1.12), mediates covalent binding of biotin to histones. Biotinylation of histones might play a role in gene silencing, cell proliferation, and DNA repair or apoptosis. Treatment of cell lines with a pharmacological concentration of biotin (10 pmol/ml) for several weeks had only a moderate impact on biotinylation of histones, whereas the biotinylation of carboxylases was strongly correlated with the biotin concentration in the culture media. A pharmacological dose of dietary biotin (100 mg/kg) has decreased the abundance of biotinylated carboxylase in rat liver. It is unclear whether an excess biotin intake would affect the biotinylation of histones, and how these changes to histones and some carboxylases are related to the detrimental effect of an excess biotin intake.

The present experiment using young rats clearly indicated that an excessive oral intake of biotin retarded the body weight gain and food intake. Judging from the results of the body weight gain and food intake, the no observed adverse effect level (NOAEL) in young rats was 0.04% in the diet, and the lowest observed adverse effect level (LOAEL) was 0.08% in the diet. Young rats in the 0.04% biotin group consumed about 6.83 g/day of their diet during days 0 to 28, the mean body weight during that period being about 177.8 g. Therefore, the

---

**Fig. 3.** Effects of Excessive Administration of Biotin on the Biotin Contents in the Muscle (A), Brain (B), Heart (C), Kidney (D), Lung (E), Spleen (F), and Testis (G).

The tissues were collected the last day of the experiment. Each bar is the mean ± SEM for 3 or 4 rats. A different superscript letter means significant difference at $p < 0.05$. 

---

Effects of Excess Biotin on Growth 2981
biotin intake was calculated as 38.4 mg/kg body weight/day. Young rats in the 0.08% biotin group consumed about 11.76 g/day during days 0 to 28, the mean body weight during that period was being about 149.7 g. Therefore, the biotin intake was calculated as 79.2 mg/kg body weight/day. Although the present study clearly showed that the 79.2 mg/kg body weight/day oral intake of biotin caused adverse effects, the present study investigated the acute, but not chronic, effects of excess biotin intake on the body weight gain, food intake, tissue weight, tissue biotin content and urinary excretion of water-soluble vitamins, and not the histopathology nor production toxicity. Furthermore, the results of the present study were obtained from a limited number of animals, four rats in each group. A further study is needed to set more accurate NOAEL and LOAEL.

A single oral administration of 20 mg of biotin or 4.5 mg intravenously to healthy adults caused no adverse effect.37) An oral intake of 1.2 mg/day of biotin by healthy adults for 14 days also did not cause any adverse effect.38) Since the data on adverse effects from a high biotin intake are not sufficient for a quantitative risk assessment, UL for biotin has not been derived in USA and Japan.15,39) The data from human studies plausibly show the low risk of several mg of biotin intake, but our

Fig. 4. Effects of Excessive Administration of Biotin on the Urinary Excretion of Thiamin(A), Riboflavin (B), 4-Piridoxic Acid (4-PIC), a Metabolite of Vitamin B6 (C), Vitamin B12 (D), Sum of the Nicotinamide Metabolites, MNA, 2-Py and 4-Py (E), Pantothenic Acid (PaA) (F), Folates (G), and Ascorbic Acid (AsA) (H).

The 24-hr urine samples were collected the last day of the experiment. Each bar is the mean ± SEM for 3 or 4 rats. A different superscript letter means significant difference at p < 0.05.
results clearly show that an excess intake of biotin increased the risk of adverse effects. A further study is therefore needed to collect enough data to set UL for biotin.

Acknowledgment

This investigation forms part of “Studies on the Dietary Reference Intakes for Japanese” (principal investigator, Katsumi Shibata) which was supported by a grant for comprehensive research on cardiovascular and life-style related diseases from the Ministry of Health, Labor and Welfare of Japan.

References


