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Original Article

Urinary excretion of biotin after intake of free and protein-bound biotin in healthy women. − A study on bioavailability of food biotin −

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Summary

Biotin functions as an essential cofactor for carboxylases in mammals. Dietary biotin exists in protein-bound and free forms and the ratio of free to total biotin varies among foods. In this study, we measured the urinary excretion of biotin in healthy women after the intake of biotin powder (the ratio of free biotin; 100%), boiled egg (40.3%) or almond (0.5%), each of which contains 50 μg of biotin, and analyzed the effect of chemical forms of biotin on biotin bioavailability. When the biotin powder was ingested, the peak of urinary excretion was shown at the point of 2 hours after intake. Meanwhile, when foods were ingested, the peak was at 4 hours. Biotin excretion after the ingestion of almond tended to be high compared with that of boiled egg, even though the ratio of free biotin in almond was almost zero. These findings indicate that the absorption of food biotin was influenced by chemical forms of biotin and other nutrients.

Introduction

Biotin is a kind of water-soluble vitamin and serves as an essential cofactor for carboxylases used in fatty acid synthesis, gluconeogenesis and metabolism of branched-chain amino acids in mammals[1, 2). Biotin deficiencies caused dermatitis, loss of hair and neurological disorder. Maternal biotin deficiency causes severe malformations in mouse fetuses[3, 4). Biotin is widely distributed in natural foods[5). Therefore, in general, it is considered that biotin deficiencies are rare in humans except for patients with a genetic defect related to biotin metabolism or individuals consuming an extremely unbalanced diet. However, it was also reported that marginal biotin deficiency was observed in pregnant women[6, 7). In pregnant mice, biotin excretion in urine was decreased on day 4 of gestation in biotin-deficient dams and on day 16 of gestation in biotin-supplemented dams[8).

Dietary biotin exists in protein-bound and free forms. The protein-bound biotin is hydrolyzed by several gastrointestinal proteases to generate biotinyl peptides, and which are further hydrolyzed by intestinal biotinidase to release free biotin before absorption[9). A portion of biotinyl peptides may be absorbed without hydrolysis by biotinidase[10). Since the ratio of protein-bound and free biotin in food is different among foods, it is thought that the bioavailability is affected by such processes as digestion, absorption, utilization, and excretion of biotin, which are different subsequent to the intake of different foods.

According to "Dietary Reference Intakes for Japanese (2015 edition)"[11), adequate intake is set for biotin. The value is 50 μg per day for adults. It was reported that the relative availability of biotin to free biotin in average diet taken by Japanese was about 80%[12). However, it is

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thought that further data is needed to clear the details of bioavailability of food biotin in humans. It is important to investigate the resulting biotin status after intake in addition to assessing dietary biotin content. In this study, we measured the urinary biotin excretion after the intake of free biotin or food biotin, and analyzed the effect of chemical forms of biotin on biotin bioavailability in fasting.

Materials and Methods

Subjects and Study design

Ten healthy women participated in the study (age 22.8 ± 0.9, body weight 51.8 ± 2.0 kg, height 158.1 ± 1.8 cm and BMI 20.7 ± 0.7 kg/m^2). They had no habit of cigarette smoking or alcohol drinking. Study design was shown in Fig. 1. The subjects began fasting from 9 p.m. the previous day. In the experimental day, they excreted the first morning urine. A whole boiled egg (225 g), dried almonds (75 g) and biotin powder for food additives were prepared as test samples. Each contains 50 μg of biotin (See Results). The only seasoning was salt. The subjects ingested one test sample each at 8 a.m. The urine was collected at 0, 2, 4, 6 and 8 hours after the intake. Water was freely available, but they did not consume meals such as breakfast and lunch on an experimental day. They were restricted from strenuous exercise during the experiment. The subjects ingested three test samples on different days. The interval of ingestion was one day or more to wash out. This study was conducted between October and November of 2013. Written consent was obtained from all subjects. The research protocol was reviewed and approved by the Ethical Committee of University of Hyogo.

Analysis of biotin

The biotin in urine was determined using a microtiter plate adaptation of a microbiological assay with *Lactobacillus plantarum* ATCC 8014 [13]. *L. plantarum* was cultured in a microtiter plate for 20 hours and determined at 610 nm. Urinary creatinine was determined on the basis of a Jaffe reaction [14]. The concentration of urinary biotin was expressed as micromoles per mole of creatinine.

The biotin in food was determined by the above bioassay. For the determination of total biotin (protein-bound plus free biotin), homogenized food solution was pretreated with 2.25 mol/L H_2SO_4 at 121°C for 60 min and neutralized with 4.5 mol/L NaOH. Free biotin was determined without an acid hydrolysis.

Statistical analysis

The values shown in the Figures of the present paper are expressed as mean ± SE. Statistical analysis of the data was performed on a personal computer using a standard statistical package (Statcel Version 2; OMS Publishing, Tokyo, Japan). Differences were considered significant if values were $P < 0.05$.

Results

Total biotin content in a boiled egg (whole) and almonds were 22.3 μg and 66.8 μg per 100 g, respectively. The ratio of free biotin to total biotin was 40.3% and 0.5%, respectively (Table 1). Therefore, 225 g of boiled egg or 75 g of almond containing 50 μg of biotin was ingested by the subjects.

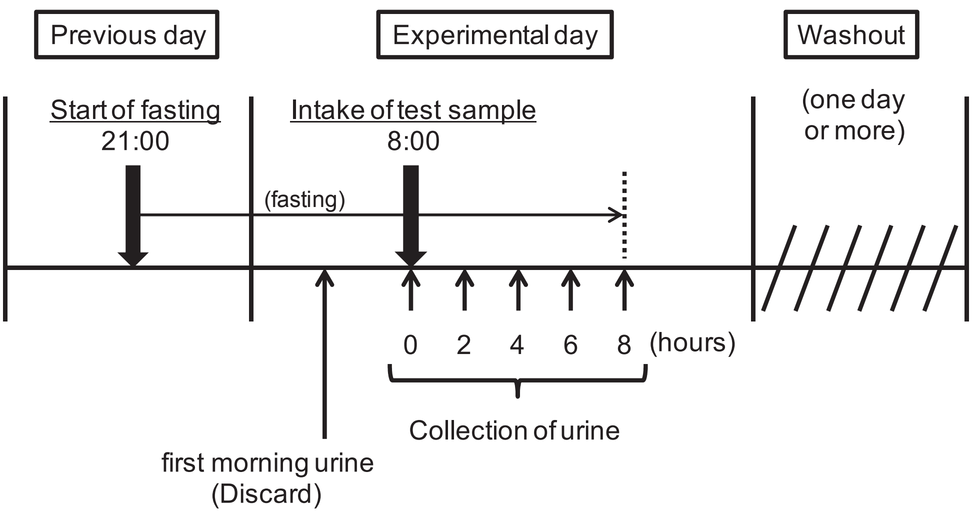

Fig. 1 Scheme of the experimental protocol

Table 1 Total and free biotin content of boiled egg and almond

	Total biotin	Free biotin	Ratio of free biotin
	(μg/100 g)		(%)
Boiled egg	22.3	9.0	40.3
Almond	66.8	0.2	0.5

The data shows the average value measured twice.

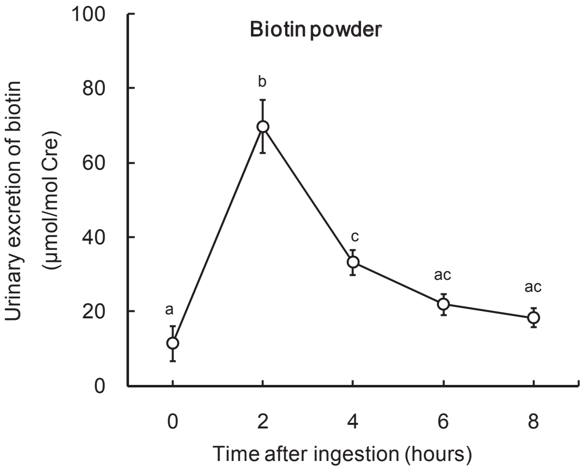

Fig. 2 Change of urinary excretion of biotin after ingestion of biotin powder
mean ± SE (n = 10).
a-c Different letters are significantly different ($P < 0.05$) (Tukey-Kramer).

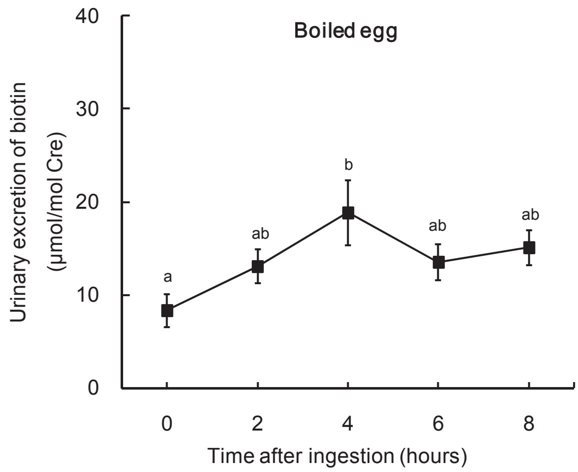

Fig. 3 Change of urinary excretion of biotin after ingestion of boiled egg
mean ± SE (n = 10).
a-b Different letters are significantly different ($P < 0.05$) (Tukey-Kramer).

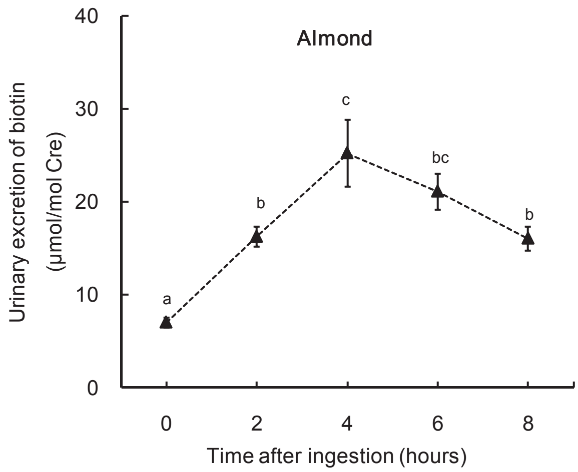

Fig. 4 Change of urinary excretion of biotin after ingestion of almond
mean ± SE (n = 10).
a-c Different letters are significantly different ($P < 0.05$) (Tukey-Kramer).

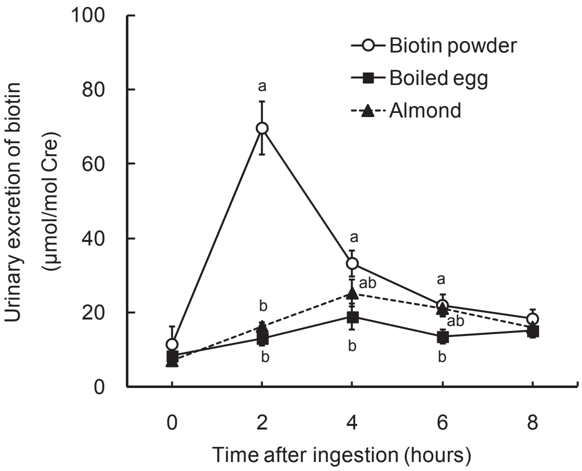

Fig. 5 Comparison among time course of urinary excretion of biotin
The values are the same of Figs. 2-4. This figure shows to analyze the difference of the time course of each experience.
mean ± SE (n = 10).
a-c Different letters are significantly different at the same time point ($P < 0.05$) (Tukey-Kramer).

Time courses of urinary excretion of biotin after ingestion of biotin powder, boiled egg and almond were shown in Figs. 2 - 4. When biotin powder was ingested, the peak (69.6 μmol/mol Cre) was at 2 hours and the level was significantly higher than that at the point of 0 hour (11.4 μmol/mol Cre). After that, the level decreased to the basal level after 6 hours. In contrast, when the foods such as boiled egg and almonds were ingested, the peak was at 4 hours (18.8 and 25.2 μmol/mol Cre, respectively). In the case of almonds, the urinary levels were significantly higher even after 6 hours compared to that at 0 hour.

Fig. 5 showed the analysis of the difference among time courses of excretion after intake of the three samples. At the point of 2 hours, the intake of biotin powder was significantly higher than that of two test foods. There was no significant difference between the urinary excretion after the intake of boiled egg and almond.

Discussion

In this study, biotin powder was used as 100% free biotin. Boiled egg and almond were used as a representative of foods in which the ratio of free to total biotin is the moderate and small degree, respectively. In addition, since eggs contribute highly to biotin intake[15), the metabolism of biotin from boiled egg is interesting.

When 50 μg of the biotin powder was ingested by the subjects, the peak of the urinary biotin was observed at the point of 2 hours after ingestion (Fig. 2). In contrast, when boiled egg or almond in the common form to eat was ingested, the peak was at 4 hours after ingestion even though the total biotin content was equal in biotin powder (Figs. 3 and 4). These results indicate that free biotin was relatively rapidly absorbed and excreted in urine, whereas biotin in food, which consists of free and protein-bound biotin, was relatively slowly absorbed since it was necessary that protein-bound biotin was hydrolyzed to free biotin by gastrointestinal proteases and biotinidase prior to absorption. Also, some kinds of chemical component in food and the process from intake to digestion might be influenced to absorption of biotin.

Since free biotin was 40.3% of total biotin in boiled egg, the peak derived from free biotin was expected to be detected. However, such a phenomenon did not occur, suggesting that absorption of free biotin "in food" might be influenced by other food components such as protein, lipids or others. Incidentally, since the egg was boiled, avidin in egg white, which strongly binds biotin, was estimated to denature and lose this feature.

Although the ratio of free biotin in almonds was rather lower than in boiled egg, when almonds were ingested, the change of urinary biotin was not significantly but tended to be higher compared to when boiled egg was ingested (Fig. 5). The result might be related to a sodium-dependent multivitamin transporter (SMVT) involved in the absorption of biotin[16)]. It was reported that SMVT binds not only biotin but also pantothenic acid and lipoic acid and transport their vitamins to enterocyte[17, 18)]. According to Standard Tables of Food Composition in Japan - 2010 -[19)], pantothenic acid content in boiled egg and almond are 3.0 and 0.4 mg per intake of this experiment, respectively. Therefore, it is possible that when boiled egg was ingested, absorption of biotin was antagonized by pantothenic acid, compared with when almond was ingested.

According to the studies using chicken and other animals, bioavailability of biotin in different foods varies from almost nothing to complete utilization[20, 21)]. However, studies of the bioavailability of food biotin to humans have been conducted only scarcely. In this study, we measured the urinary excretion of biotin after the intake of foods and biotin powder, and examined the difference of bioavailability between protein-bound and free biotin. Though the limitation of this study is not able to eliminate the influence of food components other than biotin, it is thought that this study contributes in part when nutritional index of biotin is established.

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