Effects of B-Group Vitamin Administration on Daily Change in Urine 2-Oxo Acids in Young Japanese Women

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(Received August 27, 2020)

Summary B-group vitamins are required in amino acid catabolism, and recent findings suggest that urine 2-oxo acids, catabolites of amino acid, could be functional biomarkers indicating the nutritional status of B-group vitamins. To clarify the relationship between B-group vitamins and urine 2-oxo acids, we investigated the effects of B-group vitamin administration on daily changes in urinary amounts of 2-oxo acids in humans. Twenty-nine young Japanese women collected 24-h urine samples for 8 d, and took B-group vitamins for 7 d beginning on the second day of urine collection. The participants were divided into three groups on the basis of the amounts of total branched-chain 2-oxo acids, 2-oxoglutaric acid, 2-oxoadipic acid, and pyruvic acid excreted in urine. In the upper tertile, but not the middle and lower tertiles, each urine 2-oxo acid decreased from the first day of vitamin administration, and completely decreased to a normal level on the second day of administration. These results indicate that administration of B-group vitamins immediately affects 2-oxo acid metabolism in some young Japanese women. Thus, urinary 2-oxo acids could be useful and functional biomarkers for B-group vitamin status.

Key Words 2-oxo acids, urine, biomarker, intervention study, B-group vitamins

When giving nutritional guidance, assessment of nutritional status is important. Blood concentration and urinary excretion have been measured to evaluate B-group vitamin nutritional status (1–4). Because the B-group vitamins are distributed via the bloodstream and the excess excreted to urine, blood concentrations and urinary excretions reflect the intake and body stores of these vitamins. However, these measurements cannot be used to evaluate their physiological functions such as coenzyme levels and enzyme activities required coenzymes.

We have reported the possibility of urine 2-oxo acids as a novel, noninvasive biomarker to evaluate the functions of B-group vitamins (5–7). 2-Oxo acids are catabolites of amino acids, and the B-group vitamins are required to produce and catabolize 2-oxo acids. Animal studies show that deficiency in vitamin B1, vitamin B6, or pantothenic acid increases the urinary amount of some 2-oxo acids, and the profiles of urine 2-oxo acids differ between types of vitamin deficiency (5). Additionally, streptozotocin-induced diabetic rats show greater amounts of 2-oxo acids in urine (6). One-third of young Japanese women show high amounts of urine branched-chain 2-oxo acids, and urinary excretion of these was shown to be decreased by administration of B-group vitamins for 7 d (7). These findings suggest a relationship between B-group vitamin nutritional status and urine 2-oxo acids. Although urine branched-chain 2-oxo acids are well known to use as biomarker to screen urine Maple syrup disease patients (8), only our group has tried to establish urine 2-oxo acids as a biomarker to evaluate the functions of B-group vitamins. Therefore, only a few findings about relationships between urine 2-oxo acids and B-group vitamin status is available, and the number of days and the amount and type of B-group vitamins that can decrease urine 2-oxo acids remain to be determined. In the present study, we investigated the effects of B-group vitamin administration on daily changes of urine 2-oxo acids in humans to clarify the relationship between B-group vitamins and urine 2-oxo acids.

Materials and Methods

The study was conducted from June to December 2018, and the protocol was approved by the Ethics Committee of The University of Shiga Prefecture (approval number: 653). The study was conducted in accordance with the guidelines set out in the Declaration of Helsinki. All participants provided written informed consent to participate in the study after being informed of the study protocol and purpose.
Chemicals. 2-Oxoisovaleric acid (molecular weight [MW]=116.1) was purchased from Fujifilm Wako Pure Chemical Corporation Ltd. (Osaka, Japan). 2-Oxo-3-methylvaleric acid (MW=130.1), 2-oxo-4-methylvaleric acid (MW=130.1), 2-oxoglutaric acid (MW=146.1), 2-oxoadipic acid (MW=160.1), and pyruvic acid (MW=88.1) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were of the highest purity available from commercial sources.

Participants. Female Japanese students were recruited from The University of Shiga Prefecture. All participants were non-smokers and passed a standard medical examination at the university. Participants diagnosed with a cold or influenza and those who had taken multivitamin supplements at least once during the previous month were excluded. Of the 30 apparently healthy female Japanese students aged 20–22 y who participated in the study, 1 caught a cold during the study, and 29 completed the study.

Study design. All participants took one tablet of a commercially available B-group vitamin (Eisai Co., Tokyo, Japan) each morning and night for 7 d. The participants collected 24-h urine samples for 8 d beginning on the day before the first B-group vitamin tablet was taken. The tablet contained 10 mg of thiamine nitrate, 19 mg of sodium riboflavin phosphate ester, 25 mg of pyridoxine hydrochloride, 20 mg of nicotinamide, and 10 mg of calcium pantothenate. Participants freely consumed food during the study. For assessment of dietary intake, participants completed a brief, self-administered diet-history questionnaire on food intake quantities and consumption frequency per meal over the previous month (9). Participants asked to collect 24-h urine samples a month after the cessation of the administration, and 14 participants collected urine samples.

Measurement of 2-oxo acids and B-group vitamins in urine. Urine 2-oxo acids (2-oxoisovaleric acid, 2-oxo-3-methylvaleric acid, 2-oxo-4-methylvaleric acid, 2-oxoglutaric acid, 2-oxoadipic acid, and pyruvic acid) were determined by one-way analysis of variance (ANOVA) with Bonferroni’s post hoc test. Repeated one-way ANOVA with Bonferroni’s multiple comparison test was used to assess the time-dependent change of urine 2-oxo acids and vitamins between and among the groups. A p-value <0.05 was considered statistically significant. GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) was used for all analyses.

Results and Discussion
The basic characteristics of the 29 participants are shown in Table 1. Their physical characteristics—height, body weight, and body mass index—were similar to those reported for Japanese women aged 20–29 y in The National Health and Nutrition Survey in Japan in 2018 (15). Their intakes of energy, macronutrients,
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and B-group vitamins were also similar to reported values (15). Urine thiamin, riboflavin, and 4-PIC on the day before vitamin administration were 1.1±1.2, 0.8±2.3, and 4.8±3.8 μmol/d, respectively, corresponding with previous studies (7, 16). Therefore, the participants were considered typical of young women in Japan.

Figure 1 shows the daily changes of urine 2-oxo acids in all participants. Administration of B-group vitamins significantly decreased urinary amounts of total branched-chain 2-oxo acids from the first day of administration, decreasing by 50% on the second day (p<0.05) (Fig. 1A). In contrast, other urine 2-oxo acids, including 2-oxoglutaric acid, 2-oxoadipic acid, and pyruvic acid, did not change with administration of B-group vitamins (Fig. 1B, C, D).

Because our previous study showed that one-third of participants excreted higher amounts of urine 2-oxo acids (7), we divided the participants into three groups on the basis of urine amounts of 2-oxo acids on the first day of urine collection. Physical parameters, intakes of energy, macronutrients, and B-group vitamins, and urine B-group vitamin amounts did not differ among tertiles based on any urine 2-oxo acid. Administration of B-group vitamins rapidly decreased urine total branched-chain 2-oxo acids in the upper tertile, and also decreased that on days 2, 4 and 7 in the middle tertile (Fig. 2A). On the other hand, administration of B-group vitamins failed to affect urine total branched-chain 2-oxo acids in the lower tertile. These results are consistent with our previous findings that 1-wk administration of B-group vitamins decreased urine branched-chain 2-oxo acids in the upper and middle tertiles (7).

Urine 2-oxoglutaric acid, 2-oxoadipic acid, and pyruvic acid decreased to 45–65% from the first or second day of vitamin administration in the upper tertile (p<0.05), but not in the middle or lower tertiles (Fig. 2B, C, D). Urine 2-oxoglutaric acid and 2-oxoadipic acid in the upper tertile were still two times higher than in the lower tertile during administration of B-group vitamins (Fig. 2B, C).

We also collected urine one month after the cessation of the administration from 14 participants, and measured urine 2-oxo acids. Urine total branched-chain 2-oxo acids, 2-oxoglutaric acid, 2-oxoadipic acid and pyruvic acid from days 0 to a month after administration were 8.46±4.26 to 8.79±3.41, 249±121 to 271±142, 37.7±34.6 to 30.1±16.3 and 85.4±42.0 to 83.1±39.1 μmol/d, respectively (n=14). These results showed that urine total branched-chain 2-oxo acids reverted to the basal level in a month without B-group vitamin intake.

Larger amount of urine 2-oxo acids are considered to reflect larger unused 2-oxo acids in the body. Branched-chain 2-oxo acids are derived from branched-chain amino acids, and 2-oxoadipic acid is derived from lysine and tryptophan. Pyruvic acid is a product of glycolysis and transamination from alanine, and 2-oxoglutaric acid is an intermediate of the tricarboxylic acid (TCA) cycle and a product of transamination from glutamate. That is branched-chain 2-oxo acids are directly metabolized from respective branched-chain amino acids, and other 2-oxo acids are affected by several factors such as glycolysis, TCA cycle and transamination. In the present study, urine branched-chain 2-oxo acids respond to administration of B-group vitamins well, and other 2-oxo acids still showed higher excretion in the upper tertile compared to the lower tertile. One plausible explanation is that several factors masks effect of B-group vitamins on 2-oxo acids metabolism except for branched-chain 2-oxo acids.

On the basis of these biochemical aspects, intake of excess protein, carbohydrate, or fat may enhance production of 2-oxo acids. However, we identified no relationship between urine 2-oxo acids and habitual macronutrient intakes in the present or previous study (7), and other factors may regulate the amount of urine 2-oxo acids. Branched-chain 2-oxo acids, 2-oxoglutaric acid, 2-oxoadipic acid, and pyruvic acid are metabolized by branched-chain 2-oxo acid dehydrogenase, 2-oxoglutarate dehydrogenase, 2-oxoadipate dehydrogenase, and pyruvate dehydrogenase, respectively. Any dehydrogenase complex consists of E1, E2, and E3 subunits, and they need four types of coenzymes: thiamin diphos-
phate (ThDP, functional form of vitamin B₆), flavin adenine dinucleotide (FAD, functional form of vitamin B₁₂), nicotinamide adenine dinucleotide (NAD⁺, functional form of niacin), and coenzyme A (CoA, functional form of pantothenic acid) (17–19). In the present study, one-third of participants excreted higher amounts of urine 2-oxo acids, and administration of B-group vitamins (including vitamin B₁, vitamin B₂, niacin, and pantothenic acid) decreased urine 2-oxo acid amounts to the level of the lower tertile within 2 d. Urine B-group vitamin amounts increased markedly with B-group vitamin intake, and reached a plateau in 2 d (data not shown). These results suggest that saturation of B-group vitamins in body stores promotes immediate metabolism of 2-oxo acids. One plausible mechanism is that the participants might have a minor gene mutation in some 2-oxo acid dehydrogenase complex and thus the increase in B-group vitamin coenzymes might improve activity of 2-oxo acid dehydrogenase.

The present study has two limitations. First, participants were limited to healthy Japanese women (20–22 y). It is not known whether age, sex, and diseases affect urine 2-oxo acids in humans. A few animal studies have shown that B-group vitamin deficiency and streptozotocin-induced diabetes increased urine amounts of 2-oxo acids in rats (5, 6). Second, in the present study, we did not determine which type of B-group vitamin was involved in which 2-oxo acid metabolism. Therefore, more data are required before urine 2-oxo acids can be established as a biomarker in humans.

In summary, one-third of young Japanese women showed high amounts of urine 2-oxo acids; in these participants, administration of B-group vitamins decreased levels of urine 2-oxo acids from day 1 of vitamin administration, reaching normal levels within 2 d. Habitual intake of energy, macronutrients, and B-group vitamins did not differ among participants in the different tertiles. These results suggest that saturation of B-group vitamins immediately promotes 2-oxo acid metabolism, and that some people need higher intakes of B-group vitamins to maximize the vitamins’ nutritional effect. Since 2-oxo acids are intermediates of amino acid metabolism pathway, glycolysis or TCA cycle, administration of B-group vitamins may improve energy metabolism in those who show higher urine 2-oxo acids. Future studies will clarify the amount and combination of B-group vitamins the most effective in decreasing urine 2-oxo acids in respective persons and investigate the mechanism underlying the effect on 2-oxo acids metabolism. These studies may establish urine 2-oxo acids as useful and functional biomarkers for B-group vitamins.

Authorship
Research conception and design: MK, KS and TF; experiments: MH and SH; statistical analysis of the data: MH, SH and TF; interpretation of the data: MH, SH, MK and TF; writing of the manuscript: MH and TF.

Disclosure of state of COI
We have no conflict of interests to declare.

Acknowledgments
We thank Louise Adam, ELS(D), from Edanz Group (https://en-author-services.edanzgroup.com/ac) for editing a draft of this manuscript.

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