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Urinary Water-Soluble Vitamins as Potential Nutritional Biomarkers to Assess Their Intakes

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Abstract

To determine micronutrient intake by dietary assessment is difficult because of high variations in habitual micronutrient intake. A nutritional biomarker can be an indicator of nutritional status with respect to intake or metabolism of dietary constituents. Recent validation studies have developed the urinary compounds as nutritional biomarkers to estimate nutrient intakes, and urinary nitrogen and sodium have been well established as nutritional biomarkers. Recent studies have conducted to establish urinary water-soluble vitamins as nutritional biomarkers to assess their intakes, and made the following findings to contribute to the establishment and effective use of urinary water-soluble vitamins as potential nutritional biomarkers. Only urinary vitamin B_{12} content reflects urine volume but not its intake. Eight of nine water-soluble vitamin levels in 24-hr urine increase in dose-dependent-manner, and are strongly correlated with vitamin intakes. Each urinary water-soluble vitamin level, except for vitamin B_{12}, is positively correlated with the mean intake over the recent 2-4 days in free-living children, young and elderly. These findings suggest that urinary water-soluble vitamins can be used as nutritional biomarkers to assess their mean intakes in groups. Based on previous findings, the reference values for urinary water-soluble vitamins are proposed to show adequate nutritional status.

Keywords: Water-soluble vitamins; Cross-sectional study; Intervention study; Urine; Vitamin intake; Free-living; Human subjects

Abbreviations: BMI: Body Mass Index; DRIs: Dietary Reference Intakes; TC: Transcobalamin

Introduction

To estimate nutrient intake and to determine nutritional status are important to maintain one's health. Although dietary assessment can provide approximate intake, this approach often makes misreporting, and can't determine nutritional status. Especially, to determine micronutrient intake by dietary assessment is difficult because of high variations in habitual micronutrient intake. A nutritional biomarker can be an indicator of nutritional status with respect to intake or metabolism of dietary constituents. The nutritional biomarkers can be designated into one or more of three categories, 1) a means of validation of dietary instruments, 2) surrogate indicators of dietary intakes, or 3) integrated measures of nutritional status for a nutrient [1]. Recent validation studies have developed the urinary compounds as nutritional biomarkers to estimate nutrient intakes. For example, 24-hr urinary nitrogen has been established as a biomarker for protein intake [2], same as urinary potassium and potassium intake [3], and urinary sugars for sugar intake [4].

Water-soluble vitamins are absorbed from the digestive tract after ingestion, stored in the liver, delivered to peripheral, and then excreted to urine [5]. Urinary water-soluble vitamins or their metabolites decrease markedly as vitamin status declines, and they are affected by recent dietary intake [5]. Urinary excretion of water-soluble vitamins such as thiamin, riboflavin and niacin has been used for setting Dietary Reference Intakes (DRIs) in USA and Japan [5,6]. However, only a single study had investigated urinary vitamin as a possible marker for intake until 2007. Individuals' 30-day means of thiamin intake are highly correlated with their mean 24-hr urine thiamin levels under strictly controlled condition, showing 24-hr urinary thiamin as a useful marker for thiamin intake under strictly controlled conditions [7]. Although pharmacological dose of water-soluble vitamin intake such as vitamin B_2 [8], nicotinamide [9] and biotin [10] dramatically increase urinary vitamin levels, a few study had studied about the relationship between several oral dose correspond to dietary intake and urinary excretion of vitamin C [11,12].

Recent studies have conducted to establish urinary water-soluble vitamins as nutritional biomarkers to assess their intakes. In the present review, recent findings from intervention and cross-sectional studies are described to contribute to the establishment and effective use of urinary water-soluble vitamins as potential nutritional biomarkers. Furthermore, the reference values for urinary water-soluble vitamins are proposed to show adequate nutritional status based on the findings.

Intervention Studies

Factors affecting the urinary excretions of water-soluble vitamins

It is well known that urinary excretion of these vitamins varied among subjects more than blood levels did. One possible explanation is that one or more of several factors such as nutrient requirements, energy expenditure, tissue turnover, intestinal absorption, kidney reabsorption, and physical characteristics differ between individuals. In fact, urinary excretion of vitamin B_1 is varied with the urine volume [13], and furosemide-induced diuresis increases vitamin B_1 excretion rate [14]. Physical characteristics also affect the amount of urinary compounds. For example, individuals excreting higher urinary nitrogen had greater weight and body mass index (BMI) than those excreting

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average or lower nitrogen [15], and creatinine clearance is positively correlated with BMI [16]. In this context, the physical characteristics and urine volume may affect urinary excretion of B-group vitamins. There is a report that urinary excretion of B-group vitamins was measured in free-living, healthy human subjects, and the correlations were determined between each of the urinary B-group vitamins and factors such as physical characteristics and urine volume [17].

In the report, 24-hr urine samples were collected from 186 free-living Japanese females aged 19–21 years, and 104 free-living Japanese elderly aged 70–84 years, and correlations were determined between urinary output of each B-group vitamin and body height, body weight, body mass index, body surface area, urine volume, and urinary creatinine. Only urinary excretion of vitamin B_{12} showed strong correlation with urine volume in both young female and elderly subjects. All factors such as urine volume, urinary creatinine and physical characteristics such as body height, body weight, BMI and body surface area showed weak or no correlations with other 7 urinary B-group vitamins including thiamin, riboflavin, pyridoxal metabolite 4-pyridoxic acid, sum of nicotinamide metabolites, pantothenic acid, folate and biotin. Orally administration of 1.5 mg cyanocobalamin, which is 500-fold higher daily intake, increased vitamin B_{12} content in the urine by only 1.3-fold, and urinary vitamin B_{12} was always strongly correlated with urine volume (Figure 1). Vitamin B_{12} is different from other B-group vitamins with respect to main excretion route, which is through the bile, and <10% of the total loss of vitamin B_{12} from the body is through urine [18]. These results suggest that the change in the level of urinary vitamin B_{12} is too small to evaluate intake of vitamin B_{12}, and thus urinary vitamin B_{12} was unavailable to be used as biomarker for estimation of its intake. To excrete vitamin B_{12} into urine, vitamin B_{12} binds to carrier protein transcobalamin (TC) in serum [19], the TC–vitamin B_{12} complex is filtered in the glomeruli, and the proximal convoluted tubule reabsorbs this complex via a receptor-mediated system [20]. Megalin is an essential receptor for reabsorption of the TC–vitamin B_{12} complex in the proximal tubule [21], binds to the TC–vitamin B_{12} complex with an estimated affinity (K_d) of ~183 nmol/L [22]. This high affinity may explain why urinary loss of vitamin B_{12} is very low. However, little is known about how water regulation mediated by regulatory factors such as aquaporin, vasopressin and angiotensin is linked to reabsorption of vitamin B_{12}.

Determination of urinary water-soluble vitamins as biomarkers for evaluating its intakes under strictly controlled conditions

As mentioned above, it is well known that pharmacological dose of water-soluble vitamin intake dramatically increase urinary vitamin levels, but a few study had studied about the relationship between several oral dose correspond to dietary intake and urinary excretion of vitamin C [11,12]. There is also a report to determine whether urinary levels of water-soluble vitamins and their metabolites can be used as possible markers for estimating their intakes in the intervention study [23]. In the report, six female Japanese college students were given a standard Japanese diet in the first week, same diet with synthesized water-soluble vitamin mixture as the diet as approximately one-fold vitamin mixture based on DRIs for Japanese in the second week, with three-fold vitamin mixture in the third week, and six-fold mixture in the fourth week. The 24-hr urine was collected on each week, and the relationships were determined between oral dose and urinary vitamin levels. All urinary vitamin and their metabolites levels except vitamin B_{12} increased linearly in a dose-dependent manner, and highly correlated with vitamin intake (r = 0.959 for vitamin B_1, r = 0.927 for vitamin B_2, r = 0.965 for vitamin B_6, r = 0.957 for niacin, r = 0.934 for pantothenic acid, r = 0.907 for folic acid, r = 0.962 for biotin, and r = 0.952 for vitamin C; Figure 2). These findings show that water-soluble vitamin and their metabolite levels in 24-hr urine reflect the vitamin intakes under strictly controlled conditions.

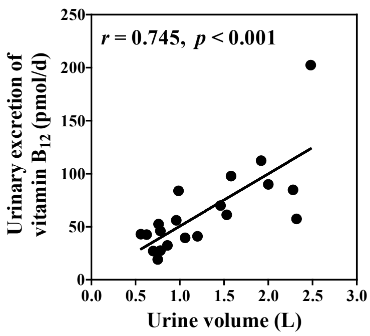

Figure 1: Correlations between urinary vitamin B_{12} and urine volume [17]. The 24-h urine was collected from 20 Japanese adults, and Pearson's correlation coefficients were determined between urinary vitamin B_{12} and urine volume.

Humans can synthesize the vitamin nicotinamide from tryptophan in the liver, and the resultant nicotinamide is distributed to non-hepatic tissues. The purpose of the synthetic pathway in the liver is not the supply of NAD^+ but the supply of nicotinamide for non-hepatic tissues. The conversion pathway of nicotinamide from tryptophan is affected by various nutrients [24-27], hormones [28,29], exercise [30] and drugs [31-34], based on data concerning the urinary excretion of metabolic intermediates in the tryptophan–nicotinamide pathway. However, the intervention study showed that administration of nicotinamide did not affect de novo nicotinamide synthesis from tryptophan [35].

Cross-sectional studies: Determination of urinary water-soluble vitamins as biomarkers for evaluating its intakes in free-living subjects

The intervention study showed that urinary water-soluble vitamin levels are correlated highly with their intake in a strictly controlled environment [23]. Performance of a study under a free-living environment without any interventions is the next step to confirm the applicability of methods using a biomarker. In this context, free-living healthy subjects who were 216 university dietetics students aged 18-27 years, 114 Japanese elementary school children aged 10-12 years and 37 Japanese elderly females aged 70–84 years were participated to the cross-sectional studies [36-38]. The subjects performed 4-day dietary assessment by recording all food consumed during the consecutive 4-day period with a weighed food record, and collected 24-hr urine samples on the fourth day. The results showed that the correlation between the urinary excretion and the dietary intake on the same day as urine collection was highest compared with the correlations on other days in each generation. Moreover, the correlations between the urinary excretion and the mean dietary intakes during the recent 2–4 days showed higher correlations, except for vitamin B_{12}, than those for daily intakes (Table 1). However, these correlations ranged from 0.27 to 0.59, and these modest correlations were not enough to use urinary vitamins as biomarkers to estimate their intakes in individuals. Several factors are known to affect water-soluble vitamin metabolism. For example, alcohol, carbohydrate and physical activity are expected to affect vitamin B_1 metabolism [39-41]; bioavailability of pantothenic acid in food is half that of free pantothenic acid [42]; and the single

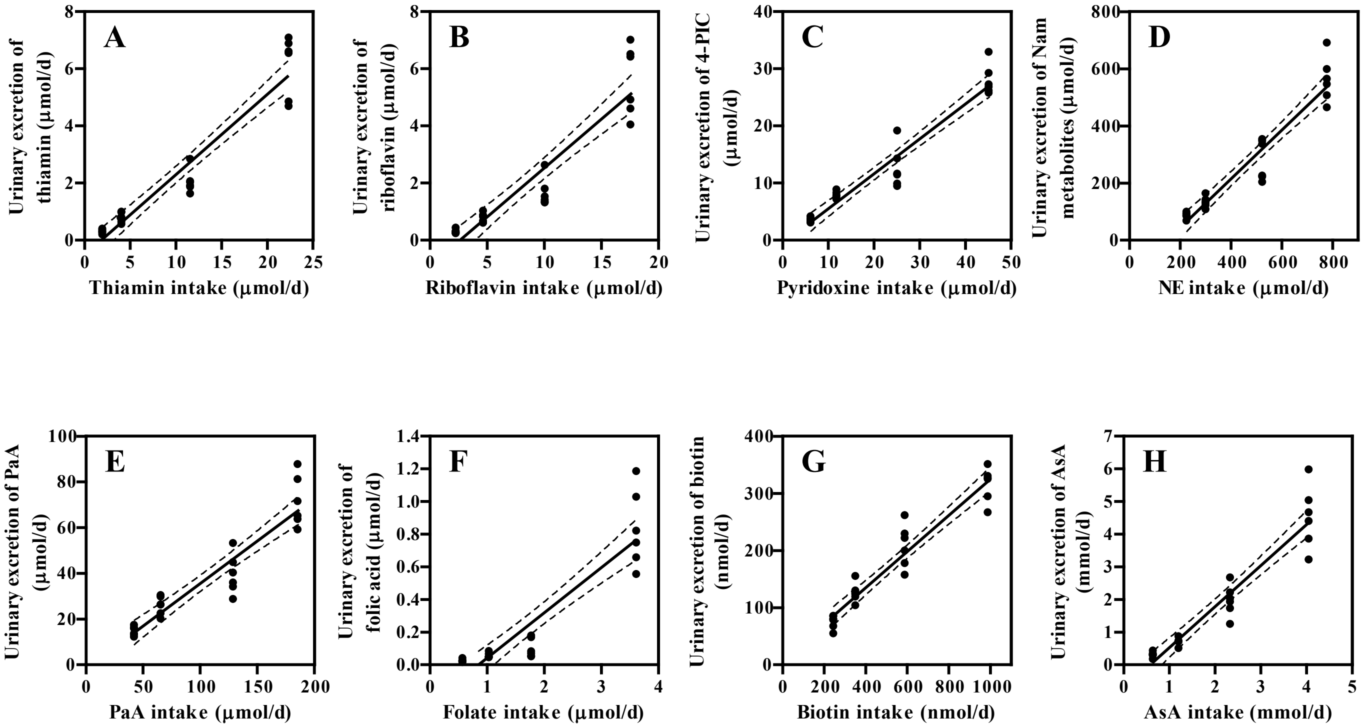

Figure 2: Regression and 95% CI of oral dose and urinary excretion of vitamin B_1 (A), vitamin B_2 (B), vitamin B_6 (C), niacin (D), pantothenic acid (E), folate (F), biotin (G) and vitamin C (H) [23]. Values are individual points of six subjects in each dose. 4-PIC signifies 4-pyridoxic acid, a catabolite of vitamin B_6 vitamers, the Nam metabolites does the total amount of nicotinamide metabolites, MNA, 2-Py and 4-Py, the PaA does pantothenic acid, and the AsA does ascorbic acid.

Vitamins	24-h urinary excretion of vitamin[a]	3 days mean vitamin intake[b]		Recovery rate[d] (%)	Mean estimated vitamin intake[e]		
	mean ± SD	mean ± SD	r^c	mean ± SD	mean ± SD	r^f	% ratio[g]
Vitamin B_1	0.425 ± 0.286 (μmol/d)	2.40 ± 0.73 (μmol/d)	0.42***	17.8 ± 11.4	2.38 ± 1.61 (μmol/d)	0.40***	100%
Vitamin B_2	0.382 ± 0.321 (μmol/d)	3.05 ± 0.83 (μmol/d)	0.43***	12.4 ± 10.0	3.08 ± 2.59 (μmol/d)	0.38***	101%
Vitamin B_6	3.68 ± 1.31 (μmol/d)	5.58 ± 1.62 (μmol/d)	0.40***	69.6 ± 28.6	5.29 ± 1.88 (μmol/d)	0.40***	95%
Vitamin B_{12}	0.028 ± 0.018 (nmol/d)	3.32 ± 2.60 (nmol/d)	0.02	1.4 ± 1.5	2.04 ± 1.33 (nmol/d)	0.06	61%
Niacin	---	95.4 ± 28.7 (μmol/d)	0.33***	---	---	---	---
Niacin equivalent	84.5 ± 28.1 (μmol/d)	192 ± 47 (μmol/d)	0.32***	45.8 ± 16.0	184 ± 61 (μmol/d)	0.33***	96%
Pantothenic acid	16.5 ± 5.2 (μmol/d)	23.9 ± 6.7 (μmol/d)	0.46***	71.6 ± 23.3	23.0 ± 7.3 (μmol/d)	0.47***	96%
Folate	23.1 ± 8.8 (nmol/d)	593 ± 243 (nmol/d)	0.27**	4.3 ± 1.9	540 ± 206 (nmol/d)	0.24**	91%
Vitamin C	139 ± 131 (μmol/d)	478 ± 267 (μmol/d)	0.42***	31.3 ± 29.6	446 ± 420 (μmol/d)	0.44***	93%

[a]Urinary excretion for each vitamin corresponds to thiamin for vitamin B_1, riboflavin for vitamin B_2, 4-PIC for vitamin B_6, the sum of nicotinamide, MNA, 2-Py and 4-Py for niacin equivalent, the sum of reduced and oxidized ascorbic acid and 2,3-diketogluconic acid for vitamin C.
[b]Mean dietary intake was calculated using daily dietary intake for each individual.
[c]*r* means a correlation between 24-h urinary excretion (Table 3) and mean dietary intake, for which values are denoted as *P<0.05, **P<0.01, ***P<0.001.
[d]Recovery rate was derived from 24-h urinary excretion/3-Days mean intake.
[e]Mean estimated intake was calculated using 24-h urinary excretion (Table 3) and recovery rate.
[f]*r* means a correlation between 3-day mean dietary intake and mean estimated intake, for which values are denoted as *P<0.05, **P<0.01, ***P<0.001.
[g]% ratio means a ratio between 3-day mean intake and mean estimated intake.

Table 1: Correlations mean water-soluble vitamin intakes in recent 3-days with 24-hr urinary excretion, recovery rates and mean estimated intakes in free-living Japanese young adults (n=148) in cross sectional studies [36].

nucleotide polymorphism of methylenetetrahydrofolate reductase (MTHFR) gene affects folate metabolism [43]. When estimated intake of water-soluble vitamins was calculated using mean recovery rate and urinary excretion values, estimated water-soluble vitamin intakes except vitamin B_{12} were correlated with 3-day mean intakes, and showed 91–107% of their 3-day mean intakes, except vitamin B_{12} (61-79%) (Table 1). These findings showed that urinary water-soluble vitamins reflected their dietary intake over the past few days, and could be used as biomarkers to assess their intakes in groups.

Relatively low correlations were found between urinary folate and dietary intake in the cross-sectional studies, whereas a high correlation was found in the intervention study [23]. The relatively low correlation of folate in free-living subjects may be explained by several reasons. Urinary folate excretion responds slowly to change in dietary folate intake, and is reduced significantly in people who consume a low-folate diet [44]. Some Japanese subjects consumed Japanese green tea and liver well, and these foods contain 16 μg/100 g and 1000 μg/100 g folate, respectively, in the Japanese Food Composition Table [45]. The composition of Japanese tea may vary depending on whether the extract of tea was made personally or whether it was a bottled tea beverage, because the present Japanese food composition table cannot differentiate such products. Similarly, since the food composition table only describes the composition of raw liver, an error exists between the quantity of vitamin intake obtained from the food composition table and the actual intake from cooked liver. Nutrient intakes were calculated using this food composition table which did not take account of cooking loss for the above foods, and thus this might cause potential low level of accuracy. There might be also a technical issue. Urinary intact folates were measured by a microbiological assay in the cross-sectional studies. However, folates are catabolized into *p*-aminobenzoylglutamate and the acetylated form, *p*-acetamidobenzoylglutamate, which are excreted into the urine [46].

Reference Values for Urinary Water-Soluble Vitamins

Urinary water-soluble vitamins can be used as potential biomarker not only for estimation of its intake but also evaluation for its nutritional status. The intervention study comprehensively investigated urinary water-soluble vitamin values in subjects consuming semi-purified diet with vitamin mixture for 7 days [47]. The study revealed the mean values and ranges for each water-soluble vitamin except vitamin B_{12} in the subjects with vitamin mixture based on DRIs for Japanese. Based on these results, the reference values for urinary water-soluble vitamins are proposed to show adequate nutritional status in Table 2. When urinary excretion of some vitamins is lower than the lower reference value, subject may not intake its vitamin enough to DRIs. When urinary vitamin is higher than the upper value, subject may intake its vitamin supplement. These reference values may be useful for first screening to check one's vitamin nutritional status and vitamin supplement intake.

Vitamins[a]	Reference values
Vitamin B_1	300-2400 (nmol/d)
Vitamin B_2	200-1800 (nmol/d)
Vitamin B_6	3.0-16.0 (μmol/d)
Vitamin B_{12}	---
Niacin	50-300 (μmol/d)
Pantothenic acid	10-60 (μmol/d)
Folate	15-80 (nmol/d)
Biotin	50-300 (nmol/d)
Vitamin C	150-2400 (μmol/d)

[a]Urinary excretion for each vitamin corresponds to thiamin for vitamin B_1, riboflavin for vitamin B_2, 4-PIC for vitamin B_6, the sum of nicotinamide, MNA, 2-Py and 4-Py for niacin equivalent, the sum of reduced and oxidized ascorbic acid and 2,3-diketogluconic acid for vitamin C.

Table 2: Proposed reference values for urinary water-soluble vitamins in adults.

Conclusion

Recent studies have induced great advances for urinary water-soluble vitamins as biomarkers for its intakes. Measuring urinary water-soluble vitamin levels can be the good approach for assessing dietary vitamin intake in groups, and for simply evaluation of its nutritional status in individuals. However, there is limitation for its use; urinary vitamins have not been suitable biomarker to estimate its intake in individuals yet. More accurate estimation of the dietary intake of water-soluble vitamins based on urinary excretion requires additional, precise biological information such as the bioavailability, absorption rate, and turnover rate. Next step in this type of study will be to determine whether vitamin contents in spot urine sample is used to assess water-soluble vitamin intakes in groups.

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References

1. Potischman N, Freudenheim JL (2003) Biomarkers of nutritional exposure and nutritional status: an overview. J Nutr 133: 873S-874S.
2. Bingham SA (2003) Urine nitrogen as a biomarker for the validation of dietary protein intake. J Nutr133: 921S-924S.
3. Tasevska N, Runswick SA, Bingham SA (2006) Urinary potassium is as reliable as urinary nitrogen for use as a recovery biomarker in dietary studies of free living individuals. J Nutr 136: 1334-1340.
4. Tasevska N, Runswick SA, McTaggart A, Bingham SA (2005) Urinary sucrose and fructose as biomarkers for sugar consumption. Cancer Epidemiol Biomarkers Prev 14: 1287-1294.
5. Food and Nutrition Board, Institute of Medicine (1998) Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academy Press.
6. The Ministry of Health, Labour, and Welfare (2009) Dietary Reference Intakes for Japanese, Tokyo.
7. Tasevska N, Runswick SA, McTaggart A, Bingham SA (2007) Twenty-four-hour urinary thiamine as a biomarker for the assessment of thiamine intake. Eur J Clin Nutr 62: 1139-1147.
8. Zempleni J, Galloway JR, McCormick DB (1996) Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. Am J Clin Nutr 63: 54-66.
9. Shibata K, Matsuo H (1990) Changes in blood NAD and NADP levels, and the urinary excretion of nicotinamide and its metabolism in women student after nicotinamide administration. Vitamins 64: 301-306.
10. Zempleni J, Mock DM (1999) Bioavailability of biotin given orally to humans in pharmacologic doses. Am J Clin Nutr 69: 504-508.
11. Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, et al. (1996) Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci USA 93: 3704-3709.
12. Levine M, Wang Y, Padayatty SJ, Morrow J (2001) A new recommended

dietary allowance of vitamin C for healthy young women. Proc Natl Acad Sci USA 98: 9842-9846.

13. Ihara H, Matsumoto T, Kakinoki T, Shino Y, Hashimoto R, et al. (2008) Estimation of vitamin B_1 excretion in 24-hr urine by assay of first-morning urine. J Clin Lab Anal 22: 291-294.

14. Rieck J, Halkin H, Almog S, Seligman H, Lubetsky A, et al. (1999) Urinary loss of thiamine is increased by low doses of furosemide in healthy volunteers. J Lab Clin Med 134: 238-243.

15. Bingham SA, Cassidy A, Cole TJ, Welch A, Runswick SA, et al. (1995) Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. Br J Nutr 73: 531-550.

16. Gerchman F, Tong J, Utzschneider KM, Zraika S, Udayasankar J, et al. (2009) Body mass index is associated with increased creatinine clearance by a mechanism independent of body fat distribution. J Clin Endocrinol Metab 94: 3781-3788.

17. Fukuwatari T, Sugimoto E, Tsuji T, Hirose J, Fukui T, et al. (2009) Urinary excretion of vitamin B_{12} depends on urine volume in female university students and elderly. Nutr Res 29: 839-845.

18. Shinton NK (1972) Vitamin B_{12} and folate metabolism. Br Med J 1: 556-559.

19. Allen RH (1975) Human vitamin B_{12} transport proteins. Prog Hematol 9: 57-84.

20. Birn H (2006) The kidney in vitamin B_{12} and folate homeostasis: characterization of receptors for tubular uptake of vitamins and carrier proteins. Am J Physiol Renal Physiol 291: F22-F36.

21. Birn H, Willnow TE, Nielsen R, Norden AG, Bonsch C, et al. (2002) Megalin is essential for renal proximal tubule reabsorption and accumulation of transcobalamin-B_{12}. Am J Physiol Renal Physiol 282: F408-F416.

22. Moestrup SK, Birn H, Fischer PB, Petersen CM, Verroust PJ, et al. (1996) Megalin-mediated endocytosis of transcobalamin-vitamin-B_{12} complexes suggests a role of the receptor in vitamin-B_{12} homeostasis. Proc Natl Acad Sci USA 93: 8612-8617.

23. Fukuwatari T, Shibata K (2008) Urinary water-soluble vitamin and their metabolites contents as biomarkers for evaluating vitamin intakes in young Japanese women. J Nutr Sci Vitaminol 54: 223-229.

24. Shibata K, Mushiage M, Kondo T, Hayakawa T, Tsuge H (1995) Effects of vitamin B_6 deficiency on the conversion ratio of tryptophan to niacin. Biosci Biotechnol Biochem 59: 2060-2063.

25. Shibata K, Kondo T, Yonezima M (1997) Conversion ratio of tryptophan to niacin in rats fed a vitamin B_1-free diet. J Nutr Sci Vitaminol 43: 479-483.

26. Kimura N, Fukuwatari T, Sasaki R, Shibata K (2005) The necessity of niacin in rats fed on a high protein diet. Biosci Biotechnol Biochem 69: 273-279.

27. Shibata K, Kondo T, Miki A (1998) Increased conversion ratio of tryptophan to niacin in severe food restriction. Biosci Biotechnol Biochem 62: 580-583.

28. Shibata K (1995) Effects of adrenalin on the conversion ratio of tryptophan to niacin in rats. Biosci Biotechnol Biochem 59: 2127-2129.

29. Shibata K, Toda S (1997) Effects of sex hormones on the metabolism of tryptophan to niacin and to serotonin in male rats. Biosci Biotechnol Biochem 61: 1200-1202.

30. Fukuwatari T, Shibata K, Ishihara K, Fushiki T, Sugimoto E (2001) Elevation of blood NAD level after moderate exercise in young women and mice. J Nutr Sci Vitaminol 47: 177-179.

31. Shibata K, Kondo T, Marugami M, Umezawa C (1996) Increased conversion ratio of tryptophan to niacin by the administration of chlofibrate, a hypolipidemic drug, to rats. Biosci Biotechnol Biochem 60: 1455-1459.

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32. Shibata K, Ishikawa A, Kondo T (1997) Effects of dietary pyrazinamide on the metabolism of tryptophan to niacin in streptozotocin-diabetic rats. Biosci Biotechnol Biochem 61: 1679-1683.

33. Shibata K, Fukuwatari T, Enomoto A, Sugimoto E (2001) Increased conversion ratio of niacin by dietary di-n-butylphthalate. J Nutr Sci Vitaminol 47: 263-266.

34. Fukuwatari T, Ohta M, Sugimoto E, Sasaki R, Shibata K (2004) Effects of dietary di(2-ethylhexyl)phthalate, a putative endocrine disrupter, on enzyme activities involved in the metabolism of tryptophan to niacin in rats. Biochim Biophys Acta 1672: 67-75.

35. Fukuwatari T, Shibata K (2007) Effect of nicotinamide administration on the tryptophan-nicotinamide pathway in humans. Int J Vitam Nutr Res 77: 255-262.

36. Tsuji T, Fukuwatari T, Sasaki S, Shibata K (2010) Twenty-four-hour urinary water-soluble vitamin levels correlate with their intakes in free-living Japanese university students. Eur J Clin Nutr 64: 800-807.

37. Tsuji T, Fukuwatari T, Sasaki S, Shibata K (2010) Urinary excretion of vitamin B1, B2, B6, niacin, pantothenic acid, folate, and vitamin C correlates with dietary intakes of free-living elderly, female Japanese. Nutr Res 30: 171-178.

38. Tsuji T, Fukuwatari T, Sasaki S, Shibata K (2011) Twenty-four-hour urinary water-soluble vitamin levels correlate with their intakes in free-living Japanese school children. Public Health Nutr 14: 327-333.

39. Hoyumpa Jr AM, Nichols SG, Wilson FA, Schenker S (1977) Effect of ethanol on intestinal (Na, K) ATPase and intestinal thiamine transport in rats. J Lab Clin Med 90: 1086–1095.

40. Manore MM (2000) Effect of physical activity on thiamine, riboflavin, and vitamin B_6 requirements. Am J Clin Nutr 72: 598S–606S.

41. Elmadfa I, Majchrzak D, Rust P, Genser D (2001) The thiamine status of adult humans depends on carbohydrate intake. Int J Vitam Nutr Res 71: 217–221.

42. Tarr JB, Tamura T, Stokstad ELR (1981) Availability of vitamin B_6 and pantothenate in an average American diet in man. Am J Clin Nutr 34: 1328-1337.

43. Bagley PJ, Selhub J (1998) A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci USA 95: 13217-13220.

44. Kim HA, Lim HS (2008) Dietary folate intake, blood folate status, and urinary folate catabolite excretion in Korean women of childbearing age. J Nutr Sci Vitaminol 54: 291-297.

45. Ministry of Education, Culture, Sports, Science and Technology (2007) Standard Tables of Food Composition in Japan Fifth Revised and Enlarged Edition. Tokyo.

46. Wolfe JM, Bailey LB, Herrlinger-Garcia K, Theriaque DW, Gregory JF III, et al. (2003) Folate catabolite excretion is responsible to changes in dietary folate intake in elderly women. Am J Clin Nutr 77: 919-923.

47. Shibata K, Fukuwatari T, Ohta M, Okamoto T, Watanabe T, et al. (2005) Values of water-soluble vitamin in blood and urine of Japanese young men and women consuming a semi-purified diet based on the Japanese Dietary Reference Intakes. J Nutr Sci Vitaminol 51: 319-328.