〈Review Article〉

Vitamins as markers of nutrition: What vitamers do we measure?

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Summary Nutritional assessment of vitamins is performed not only using a dietary questionnaire but by also determining the deficiency or the abundance of certain vitamins in blood or urine specimens. Every vitamin has several vitamers (defined as compounds with a molecular structure similar to that of a particular vitamin, which is comparable to "isomers" in chemical analysis). Therefore, there is a need for knowledge of the vitamers that we measure as markers of nutrition. Herein, we summarize the findings published to date on the vitamers of four vitamins (vitamin A, vitamin D, vitamin B_1, and vitamin C), as the Japanese tend to be deficient in their daily intake of these four vitamins. The physiological functions, blood concentrations, and half-lives of the vitamers were considered in this review.

Key words: Nutritional assessment, Retinol, 25-Hydroxyvitamin D, Thiamine pyrophosphate, Ascorbic acid

1. Introduction

Vitamins are essential nutrients required for a healthy life in humans. Our body cannot synthesize most of the vitamins except for niacin, menaquinone-7, and cholecalciferol. Niacin is synthesized from dietary tryptophan, and menaquinone-7 (vitamin K) is synthesized by enteric bacteria and is absorbed through the portal vein[1]. Moreover, some amount of cholecalciferol (vitamin D) is synthesized from 7-dehydrocholesterol present in the skin that was exposed to ultraviolet B radiation in sunlight at body temperature. Unfortunately, the production of

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such vitamins does not meet our daily requirement. Therefore, there is a need for vitamin intake through diet or dietary supplements.

The recent National Health and Nutrition Survey in Japan (NHNS-J), conducted in 2018, revealed that median (and/or average) intake levels of vitamin A, vitamin D, vitamin B_1, and vitamin C were apparently lower than the defined reference intake level for each vitamin[2]. However, because dietary intake assessment does not consider the magnitude of vitamin absorption in the small intestine, it is somewhat less accurate than the selected analysis of vitamins in body fluids such as blood and urine.

The Dietary Reference Intakes (DRIs) for the United States and Canada[3-6], and Japan[7] has defined the nutrient intake levels for four fat-soluble vitamins (vitamin A, vitamin D, vitamin E, and vitamin K) and nine water-soluble vitamins (vitamin B_1, vitamin B_2, vitamin B_6, niacin, pantothenic acid, folate, vitamin B_{12}, biotin, and vitamin C). All of these vitamins have several vitamers[1] (i.e., compounds with a molecular structure similar to a particular vitamin, which are comparable to "isomers" in chemical analysis), among which selected vitamers are used as a marker for nutritional assessment. Therefore, there is a need for knowledge of the vitamers that we measure as markers of nutrition. In this short review, we present an overview of the most recent evidence, including our observations on vitamers of the aforementioned four vitamins measured in body fluids such as blood and urine that can be used as a marker for assessing nutritional status.

2. Fat-soluble vitamins

Among fat-soluble vitamins, vitamin A, vitamin K, and vitamin D bind to the nuclear receptor. β-Carotene (precursor of vitamin A) and vitamin E act as an antioxidant, while vitamin K acts as a coenzyme apart from being a ligand that binds to the nuclear receptor[1]. Although vitamin A and vitamin D and their metabolites have several kinds of structural and geometrical isomers[8], we describe only major vitamers present at high concentrations in body fluids.

Vitamin A

In humans, vitamin A is present in plant foods and consumed in the form of β-carotene, which is converted to retinal. Vitamin A is also consumed as retinyl esters (mostly as retinyl palmitate) from animal foods. Although vitamin A has three major vitamers, namely, retinal, retinol, and retinoic acid, "retinol" is the vitamer exclusively measured for nutritional assessment[1,5]. Retinal is bound to opsins in visual function, and retinoic acid binds to the nuclear receptor for the regulation of genomic expression. Fundamentally, retinal (11-*cis* form) is formed from retinol in the retinal pigment epithelium of the ocular tissue[8], and thus, serum retinal concentration (mouse data) is 4% (32.2 nmol/L) that of retinol (810 nmol/L)[9]. The half-life of retinoic acid is 0.8 hr, and its serum concentration (23.2 nmol/L) is lower than that of retinol (1.05–2.80 μmol/L) in humans[10,11]. Retinol, the transporting form of vitamin A, has no physiological function; however, it behaves as the vitamin A reservoir in the plasma (Table 1). Previously, we revealed that plasma levels of retinol remain constant until the retinol stored in the liver (as retinyl esters) becomes exhausted[12]. Retinol present in circulating plasma binds to the retinol-binding protein 4 (RBP4) and becomes complexed with transthyretin as an equimolar binding of each of the three compounds. Accordingly, serum retinol levels can be nearly estimated from serum RBP4 concentrations. Holo-RBP4 bound to transthyretin exclusively exists in circulating plasma but not in tissues and has a half-life of 11–16 hr in humans[1].

Vitamin D

Vitamin D has two major vitamers: ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Vitamin D is hydrophobic; therefore, all vitamin D vitamers present in the plasma become bound to vitamin D-binding protein (DBP). Ergocalciferol is synthesized by the photolysis of plant sterols, and cholecalciferol is synthesized through the photolysis

Table 1 Selected markers of nutrition in major vitamers

	Major vitamers	Markers of nutrition	Physiological functions	Specimens*: Blood concentrations	Half-lives
Vitamin A	Retinal Retinol Retinoic acid	Retinol bound to RBP4	Transport form of vitamin A	Plasma or serum: predominantly, Retinol bound to RBP4	Retinol bound to RBP4 > Retinoic acid
Vitamin D	Ergocalciferol Cholecalciferol (D_3)	25-OH-D_3 bound to DBP	Transport form of vitamin D_3	Plasma or serum: 25-OH-D_3 > 1,25-$(OH)_2$-D_3	25-OH-D_3 > 1,25-$(OH)_2$-D_3
Vitamin B_1	Thiamine Thiamine monophosphate (TMP) Thiamine pyrophosphate (TPP) Thiamine triphosphate (TTP)	TPP	Act as coenzymes	Whole blood or RBC: TPP > TMP > Thiamine > TTP	TPP > Thiamine
Vitamin C	Ascorbic acid Dehydroascorbic acid (DHA)	Ascorbic acid	Act as coenzymes and an antioxidant	Plasma or serum: Ascorbic acid > DHA	Ascorbic acid > DHA

Abbreviations: RBP4, retinol-binding protein 4; DBP, vitamin D-binding protein; RBC, red blood cells.
*Specimens used for the measurement of predominant vitamer.

of 7-dehydrocholesterol in the skin. Ergocalciferol and cholecalciferol are converted to 25-hydroxyvitamin D_2 (25-OH-D_2) and D_3 (25-OH-D_3) by hydroxylation, respectively, in the liver, which does not retain 25-OH-D_2 and 25-OH-D_3[1]. They are transported to the kidney and further hydroxylated to the active form of vitamin D, i.e., 1,25-$(OH)_2$-D_2 and 1,25-$(OH)_2$-D_3. Both 1,25-$(OH)_2$-D_2 and 1,25-$(OH)_2$-D_3 bind to the nuclear receptors of vitamin D distributed mainly in bone osteoblasts and epithelial cells of the kidney and intestine. 25-OH-D_3 has a longer half-life in circulating plasma (2–3 wk) than 1,25-$(OH)_2$-D_3 (4–6 hr)[13]; therefore, "25-OH-D_3" reflects the body's store of vitamin D and is considered as a marker of nutritional status[6] (Table 1). The serum concentration[11] is 16–65 pg/mL for 1,25-$(OH)_2$-D_3 and 14–60 ng/mL for 25-OH-D_3. In our study[14], because the concentration of 25-OH-D_2 is less than 1.0 ng/mL, it is unsuitable for consideration as a nutritional marker. Tissues (e.g., beef kidney) contains low amounts of 25-OH-D_3, i.e., 0.9–23.3 ng/g as compared with that in plasma, although the highest concentrations are found in the kidney and the lowest in muscle meat (0.2–4.1 ng/g)[15].

3. Water-soluble vitamins

Water-soluble vitamins function as enzyme cofactors. Among water-soluble vitamins, vitamin C acts as a coenzyme for several enzymes, and additionally acts an antioxidant.

Vitamin B_1

Vitamin B_1 has four vitamers[8]: thiamine, thiamine monophosphate (TMP), thiamine pyrophosphate (TPP), and thiamine triphosphate (TTP). Plant foods are rich in thiamine, while animal foods are rich in TPP and TTP[1,3]. Thiamine phosphate esters are absorbed in the jejunum of the small intestine after hydrolysis to thiamine by intestine alkaline phosphatase. Body cells including erythrocytes contain all four forms of B_1 vitamers; however, thiamine and TMP are predominantly present in the plasma[16]. TPP and TTP have physiological functions; TPP acts as a coenzyme for various enzymes involved in the carbohydrate and amino acid metabolism. The physiological functions of TTP are not exactly known, but it is considered to have an effect on neurotransmission. Therefore, "TPP" is considered as a marker of vitamin B_1 nutriture (Table 1). TMP is reported to be a more sensitive marker of deficiency than TPP according to the catabolic pathway wherein TMP is formed exclusively from TPP[17]. Unfortunately, we have not encountered such a case thus far. Herein, the whole-blood concentration is 2–18 nmol/L for thiamine, 4–60 nmol/L for TMP, 63–229 nmol/L for TPP, and 0–4 nmol/L for TTP[18,19], while the serum concentration is 1–15 nmol/L for thiamine, 1–16 nmol/L for TMP, 0–3 nmol/L for TPP, and 0.0 nmol/L for TTP. The elimination half-life of TPP (664 min by intravenous administration) is longer than that for thiamine (96 min by intravenous administration and 154 min by

oral administration)[20].

Recently, patients are reported to have deficiency in thiamine transporter[21,22]. In these patients who manifest thiamine deficiency symptoms, mutations have been suggested in the thiamine transporter-1, encoded by the *SLC19A2* gene, which transports thiamine across the plasma membrane or another transporter, encoded by the *SLC25A19* gene, which transports TPP into the mitochondria[23]. Although genomic assay is required in these patients, we are planning to identify the former cases by comparing "thiamine and TPP levels" in the extracellular fluid (plasma) and the intracellular fluid (fluid within the erythrocytes).

However, we have reported that the nutritional status of vitamin B_1 nutriture can be estimated from urinary thiamine excretion (the end-products of TPP breakdown)[24]. Here, the thiamine-to-creatinine ratio in the first-morning urine is significantly correlated with total vitamin B_1 concentration (i.e., sum of thiamine and the other three thiamine esters: r= 0.735, $p< 0.001$), and also correlated to thiamine-to-creatinine ratio in 24-hour urine (r= 0.970, $p< 0.001$). Excretion of thiamine in the first-morning urine collected without voiding for more than 8 hr would reflect one-third of the excretion in the 24-hour urine of the previous day.

Vitamin C

Vitamin C has two vitamers: ascorbic acid and dehydroascorbic acid (DHA: the oxidized form). Japanese consume vitamin C through the intake of vegetables and fruits[2]. Fruits purchased from markets often contain DHA (10–90% of total ascorbic acid)[25]. Herein, the total ascorbic acid is the sum of ascorbic acid and DHA. Dietary ascorbic acid is absorbed through sodium-dependent vitamin C transporter type 1 (SVCT) in the intestinal epithelial cells (i.e., enterocytes), and DHA is absorbed through glucose transporters (GLUTs)[26,27], which is promptly reduced to ascorbic acid in the enterocytes[28]. Therefore, the serum concentration of DHA is as low as 0.8–1.4 mg/L when compared with that of total ascorbic acid (10.8±2.4 mg/L)[29,30]. The ascorbic acid concentration in human tissues (including white blood cells) is ca. 3-fold (337 mg/L in white blood cells) to 50-fold (50 mg/100 g in the pituitary) higher than that in the plasma[1,31]. Moreover, ascorbic acid has a half-life of 30 min in the plasma, and the ascorbic acid half-life in the body is 10-20 days[32,33]. Since the plasma levels of ascorbic acid depend on body stores and daily intake, "ascorbic acid" can be used as a marker of vitamin C nutriture[4] (Table 1).

4. Future perspective

The review on vitamers that can be used as a nutritional marker can be summarized as follows:

1. Vitamers used for nutritional marker have long half-lives and present at high concentrations in the blood or plasma compared to other vitamers that are unable for use as a nutritional marker.
2. The lipid-soluble vitamers retinol and 25-OH-D_3, which are used as a nutritional marker, exist in the transport form, both of which have no physiological functions. Plasma concentrations of these vitamers are higher than their tissue levels.
3. The water-soluble vitamers TPP and ascorbic acid, which are used as a nutritional marker, have physiological functions. Tissue concentrations of these vitamers are higher than their plasma concentrations

Further studies are needed to assess whether this tentative definition could be applicable to other vitamins that meet the daily requirement in Japanese individuals.

Conflicts of interest

The authors have no conflicts of interest.

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