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Analysis of Vitamin B_{12} in the Korean Representative Foods and Dietary Intake Assessment for Koreans

Younju Choi[1], Jiyung Kim[2], Haeng-Shin Lee[3], Cho-il Kim[3], In Kyeong Hwang[4], Hye Kyung Park[5], Tae-Hwa Kim[6], and Chang-Hwan Oh*

Department of Oriental Medical Food and Nutrition, Semyung University, Jecheon, Chungbuk 390-711, Korea
[1]Korea Food and Drug Administration, The Bureau of Risk Management, Seoul 122-704, Korea
[2]Ottogi Research Center, Anyang, Gyeonggi 431-070, Korea
[3]Korea Health Industry Development Institute, Seoul 156-800, Korea
[4]Department of Food and Nutrition, College of Human Ecology, Seoul National University, Seoul 151-742, Korea
[5]Korea Food and Drug Administration, Nutrition and Functional Food Headquaters, Seoul 122-704, Korea
[6]Institute of Safety and Quality Evaluation for Agricultural Products, Kyungpook National University, Daegu 702-701, Korea

Abstract This study was conducted to provide a vitamin B_{12} database for the representative Korean food items and to assess the dietary intake assessment of vitamin B_{12} for Koreans. The vitamin B_{12} content of 106 foods had been determined by high performance liquid chromatography (HPLC) using column switching method. Rich sources of vitamin B_{12} were meats, milk, and egg (0.3-3.4 μg/100 g). Vegetables and fruits contained vitamin B_{12} below limit of detection (LOD). The major food sources for vitamin B_{12} intake were milk (72.0%), meats (22.3%), egg (3.6%), and fishes & shellfishes (2.1%). Mean vitamin B_{12} intake of Koreans was 3.16 μg/person/day. The proportion of population with intake below estimate average requirement (EAR) and above recommended intake (RI) of vitamin B_{12} was 60.7 and 36.5%, respectively. The vitamin B_{12} intake level of young children with 1-2 years which was 834.6% of RI while the intake level of the older adults 50 years and older was only 70.0% of RI. Also, there were regional differences between urban and rural area. The population with intake below RI was larger than that with intake above RI in Korea.

Keywords: vitamin B_{12}, database, dietary intake assessment, dietary reference intake for Korean

Introduction

Vitamin B_{12} is a member of the vitamin B complex (1,2). It is unique vitamin that contains a metal ion, cobalt, and so is also known as cobalamin (2). Methylcobalamin and 5-deoxyadenosyl cobalamin are the forms of vitamin B_{12} used in human body. The form of cobalamin used in most supplements, cyanocobalamin, is readily converted to methylcobalamin and 5-deoxyadenosyl cobalamin (2,3).

Vitamin B_{12} works with folic acid in many body processes including synthesis of DNA, red blood cells and the insulation sheath (the myelin sheath) that surrounded nerve cells and facilitates the conduction of signals in the nervous system (1,4-7). Vitamin B_{12} deficiency results in impairment of the activities of B_{12}-requiring enzymes, such as L-methylmalonyl-CoA mutase (1-3) and methionine synthase (8,9).

A deficiency may still occur as a result of an inability to absorb vitamin B_{12} from food and in strict vegetarians who do not consume any foods that come from animal (5). Strict vegetarian and infants without maternal feeding should be supplied by vitamin B_{12} through nutritional supplements or infant formulas (10). The Korean Food Code Specifications for vitamin B_{12} in infant and follow-up formulas are 0.1, and 0.15 μg/100 kcal, respectively (11).

Available methods for vitamin B_{12} analysis include polarographic, spectrophotometric, and various chromatographic (such as paper, thin-layer, open column, gas, and liquid), microbiological, and radio-ligand binding procedures (12). High performance liquid chromatography (HPLC) is the one of the chromatographic analysis methods for vitamin B_{12} (13-15). The previously developed μ-HPLC using the column-switching technique, for the determination of vitamin B_{12}, was applied in this study (16-18).

Dietary reference intakes (DRIs) is the general term for a set of reference values for planning and assessing nutrient intake of healthy population (19). Korean dietary reference intakes (KDRIs) were set in 2005 by the Korean Nutrition Society (20). KDRIs of vitamin B_{12} are as follows; estimated average requirements (EAR) is 2 μg/day, recommended intake (RI) is 2.4 μg/day for adults and adequate intake (AI) is 0.2 and 0.5 μg/day for 0-5 and 6-11 months, respectively. The upper limit (UL) has not been established because it is unlikely to result in adverse health effects.

Many people have concerned vitamin B_{12} intake of Korean, nevertheless, the database of vitamin B_{12} in typical Korean diet had not been presented. Therefore, this study was carried out to produce a food vitamin B_{12} database and to assess the dietary intake of vitamin B_{12} for the Korean population using Korea Nutrition and Health Examination II (KNHANES II) data.

Materials and Methods

Reagents and apparatus Vitamin B_{12} standard and potassium phosphate monobasic were obtained from

*Corresponding author: Tel: +82-43-649-1434; Fax: +82-43-649-1759
E-mail: och35@semyung.ac.kr or changhwan@hanmail.net
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Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Standard was dissolved in 5 mM potassium phosphate solution (pH 3.0). HPLC grade solvents such as methanol, water, and chloroform were purchased from Burdick & Jackson (Fair Lawn, NJ, USA). Phosphoric acid was obtained from Merck (Frankfurter, Germany). An HPLC (Nanospace SI-2; Shiseido, Tokyo, Japan) equipped with autosampler, pump, UV detector, PDA detector, and valve system was used. Other equipment included a sonicator (JAC 2010; Jinwoo Engineering, Seoul, Korea) and a centrifuge (Hanil MF 500; Hanil Co., Seoul, Korea) were used.

Sampling Food intake data of the Korean population from the nutrition survey part of KNHANES II (21) were used to develop a food list for vitamin B_{12} analysis. High and frequent consumption foods by Koreans (n=76) and infant formula (n=1) to reflect differences in dietary intake patterns by babies age 1-3 years. Foods with a probability of being high in vitamin B_{12} (n=29) were included according to references (1,2,4) and seasonal variation was also considered. The sum weight and energy for the selected foods were 979.3 g and 1,551 kcal and it amounts to 80.6% of total food weight and 83.0% of total energy for Koreans derived from KNHANES II. Selected foods representing what is commonly consumed in Korea were purchased at large market in Seoul and Gyeonggi province, Korea and they are domestic or imported ones. Three samples for each food were purchased from June to October in 2005. The samples were grinded and dried to constant weight (150 g) and stored in airtight polyethylene bottles at −18°C.

Sample preparation Vitamin B_{12} in foods was extracted by the method in the previous report (18). Each sample (10 g) was suspended in 5 mM potassium phosphate solution and phosphoric acid (1%) was used to adjust pH 2.5. The mixture was extracted by sonication in ultrasonic bath for 10 min and the made to volume (50 mL) with phosphate solution. The middle layer was collected and chloroform (3 mL) was added to remove lipids and centrifuged at 3,000 ×g, again. The clear layer was passed through a 0.20 μm membrane filter.

HPLC analysis The switching valve HPLC system with triple column used in this study. Pump 1 was used to deliver eluent A (5 mM KH_2PO_4) at a flow rate of 500 μL/min and pump 2 was used to deliver eluent B (5 mM KH_2PO_4/MeOH) at a flow rate of 100 μL/min, respectively. A sample solution was introduced to the pretreatment column (Capcellpak MF SG80, 4.6×150 mm, 5 μm) using eluent B. By switching the valve, vitamin B_{12} is eluted from the pretreatment column and introduced to the concentration column (Capcellpak C18 UG120, 2.0×35 mm, 5 μm). Finally vitamin B_{12} adsorbed in the concentration column is introduced to the separation column (Capcellpak C18 UG120, 1.5×250 mm, 5 μm) by eluent A after switching the valve. Total analytical time was 25 min per sample and the detector was used at 361 nm.

Statistical methods To estimate dietary intake of vitamin B_{12}, the vitamin B_{12} content data from this analysis was multiplied with food consumption data from KNHANES II and the results were compared to KDRIs (20). All computations were performed using SAS for Windows version 9.1 (2001).

Results and Discussion

Analysis of vitamin B_{12} in the Korean representative foods Vitamin B_{12} content in various foods is shown in Table 1. The highest values found in meat & poultry and baby food, such as pork liver (3.4 μg/100 g), infant formula (1.8 μg/100 g), pork belly (1.5 μg/100 g), and beef loin (1.0 μg/100 g) followed by mackerel, chicken, and others. Those samples containing high content of vitamin B_{12} were all animal origin foods. Only a few foods (n=23) was found to contain vitamin B_{12} above limit of detection (LOD). Vitamin B_{12} was detected under the LOD in grains & cereal products, starches & pulses, vegetables, seaweeds,

Table 1. Vitamin B_{12} content in representative Korean diet

Foods	Content (μg/100 g)	Foods	Content (μg/100 g)
Meats & meat products (16)			
Beef, loin	1.0	*Beef, rib*	0.6
Beef, shank	0.5	*Pork, Loin*	0.7
Pork, rib	0.7	*Chicken*	0.9
Pork, belly	1.5	*Ham*	0.6
Beef, loin	0.4	*Beef, brisket*	0.7
Beef, small intestine	0.5	*Beef, intestine*	0.6
Pork, liver	3.4	*Sausage*	0.0
Duck	0.8	*Dumpling*	0.0
Fishes, shellfishes & their products (17)			
Fish cake	0.0	*Common squid*	0.0
Mackerel	0.1	*Alaska pollack*	0.5
Anchovy, boiled and dried	0.0	*Yellow croaker*	0.0
Hair tail	0.0	*Little neck clam*	0.0
Crab	0.0	*Flounder*	0.0
Shrimp	0.0	*Japanese spanish mackerel*	0.0
Octopus	0.0	*Billfish*	0.5
Tuna	0.0	*Eel*	0.0
Tuna, canned	0.6		
Milk, dairy products & egg (8)			
Milk	0.3	*Yoghurt*	0. 7
Ice cream	0.0	*Yoghurt, paste*	0.0
Infant formula	1.8	*Cheese*	0.0
Baby food	0.8	*Egg*	0.6
Grains & cereal products (14)			0.0
Starches & pulses (3)			0.0
Vegetables (19)			0.0
Seaweeds (2)			0.0
Fruits (10)			0.0
Beverages & alcoholic beverages (6)			0.0
Fats & oils (4)			0.0
Seasonings & spices (7)			0.0

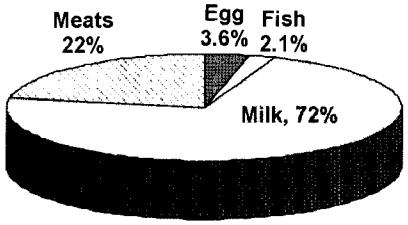

Fig. 1. The contribution of food groups for vitamin B_{12} intake.

fruits, beverages & alcoholic beverages, fats & oils, and seasonings & spices. Other study (1,22,23) showed that fruits, vegetables, grains, and grain products are devoid of vitamin B_{12} except when contaminated with fecal matter used as fertilizer (1). Though fermented soya products and seaweeds have all been proposed as possible sources of B_{12}, there was no significant vitamin B_{12} in those samples. Considering the amount of food consumption, the major food sources of vitamin B_{12} intake were milk (72%), meats & poultry (22%), egg (3.6%), and fishes (2.1%) (Fig. 1). It is known that the only reliable unfortified sources of vitamin B_{12} are meat, dairy products, and eggs (10), the result of this study establish this fact. The study of the dietary intake of folate, vitamin B_6, and vitamin B_{12} among Japanese adults showed fish/shellfish (29) was the largest source for vitamin B_6 (16-23%) and B_{12} (77-84%). It is verified that the major food of nutrient intake may be different as food intake pattern of each nation.

Dietary intake assessment of vitamin B_{12} for Koreans

This study adopted simple distribution estimate (24) to evaluate vitamin B_{12} intake. The vitamin B_{12} content database from this study was multiplied by the data of food intake distribution from 24 hr dietary recall data in KNHANES II which was conducted on anyone 1 year and older in sample households drawn by stratified multi-stage sampling methods (n=9,968). As a result, the average intake of vitamin B_{12} for total population, male and female was 3.16, 3.55, and 2.80 μg/person/day, respectively (Table 2). And the rural population showed lower intake of vitamin B_{12} than the urban population (Table 2). Results of two national surveys in US, the National Health and Nutrition Examination Survey (NHANES III 1988-1994) (25) found that most children and adults in the United States consume the recommended amounts of vitamin B_{12}; Male 4-5 μg/person/day and female is 3 μg/person/day.

As a result of assessment for vitamin B_{12} intake by age, the group from 1 to 2 years has been identified as the highest intake (7.51 μg/person/day) group of vitamin B_{12}. The intake proportions to RI were as follows; 1-2 (834.6%), 3-6 (505.0%), and 7-12 years (356.6%) groups (Table 2). It may be result from high vitamin B_{12} content in dairy products which are the favorite and major food for them. And it may be not desirable to fortify the food for infants and children under the age of elementary school. The intake proportion to RI of 13-19 and 20-29 years group were 199.0 and 119.8%, respectively. On the other hand, the intake by over 50-64 years and over 65 years group were 69.2 and 58.2% of RI (Table 2). It might be due to the fact that the Korean elderly people usually take less animal food, such as meats and milk products, than other adult

Table 2. The mean intake and the proportion to recommended intake (RI) by region and gender groups for vitamin B_{12}

	Frequency	Amount (μg/day)	Proportion (%) to RI
Area			
All area	9,968	3.16	
Metropolitan area	4,586	3.31	177.5
Urban area	3,287	3.30	187.1
Rural area	2,095	2.46	136.3
Gender			
Male	4,760	3.55	199.0
Female	5,208	2.80	150.3
Age			
1-2 years	250	7.51	834.6
3-6 years	684	5.80	505.0
7-12 years	1,088	6.04	365.6
13-19 years	968	4.63	199.0
20-29 years	1,256	2.87	119.8
30-49 years	3,406	2.26	94.2
50-64 years	1,400	1.66	69.2
≥65 years	916	1.40	58.2

Table 3. The frequency and proportion of the population at each level of vitamin B_{12} intake

	<EAR[1)]	EAR≤Vit B_{12} <RI[2)]	RI≤Vit B_{12}
Frequency	6,048	279	3,641
Proportion (%)	60.7	2.80	36.5
Mean intake (μg)	0.56	2.03	7.63

[1)]Estimated average requirements, whose vitamin B_{12} is 2 μg/day for Korean adults.
[2)]Recommended intake, whose vitamin B_{12} is 2.4 μg/day for Korean adults.

group. Dhonukshe-Rutten *et al.* (8,9) found that vitamin B_{12} status is associated with bone health in elderly women. That is, osteoporosis occurred more often among women whose vitamin B_{12} status was considered marginal or deficient than in women with a normal status. And Morris *et al.* (26) reported that in seniors with low vitamin B_{12} status was associated with anemia and cognitive impairment. Future studies on bone health and neuro-psychiatric disorders should take into account a possible role of vitamin B_{12} status in Korean elderly population because the deficiency of vitamin B_{12} seems to be in general.

Also, Stabler *et al.* (27) reported that vitamin B_{12} deficiency is present in up to 15% of the elderly population as documented by elevated methylmalonic acid with low vitamin B_{12} concentration. Oral vitamin B_{12} supplementation may be effective in lowering serum methylmalonic acid values in the elderly. However, the dose of vitamin B_{12} in most common multivitamin preparations is too low for this purpose. Therefore, it is needed that they should intake more meat and dairy products.

As KDRIs were developed in 2005, we compared the vitamin B_{12} intake level with KDRIs. The proportion of the Korean population with intake below EAR, from EAR to

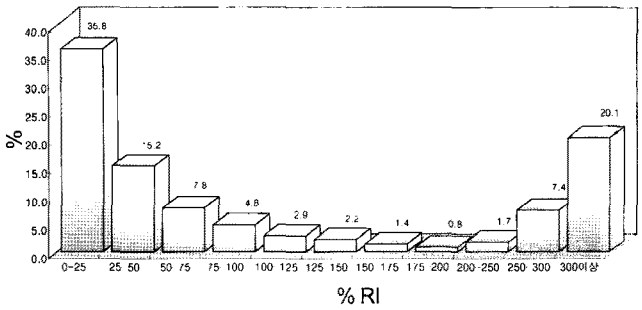

Fig. 2. The distribution of population depending on vitamin B_{12} intake comparing to recommended intake (RI).

Table 4. The correlation between vitamin B_{12} and other nutrients

Nutrients	Correlation coefficient[1)]
Energy	0.176
Protein	0.218
Fat	0.363
Sugar	0.019
Calcium	0.038
Phosphorus	0.241
Ferrous	0.004
Sodium	0.060
Potassium	0.118
Vitamin A	0.055
Vitamin B_1	0.209
Vitamin B_2	0.404
Niacin	0.100
Vitamin C	0.037
Percent of energy from fat	0.407

[1)]All values were significant at $p<0.001$.

RI, and above RI of vitamin B_{12} was 60.7, 2.80, and 36.5 %, respectively (Table 3) and the intake level of each group as follows: 0.56, 2.03, and 7.63 μg/person/day, respectively. Because of limited sources of vitamin B_{12}, the person who does not like those foods derived from animal might intake vitamin B_{12} below the recommended level.

Figure 2 shows that the proportion of the population with intake below RI was larger than that with intake above RI. That is, the population below 100% of RI was 63.5% and above 100% of RI was 36.5%. The reason of high rates of vitamin B_{12} insufficiency is thought to be that the grains and vegetables, the major foods for Korean, could not contribute to vitamin B_{12} intake. But the average intake of total population (Table 2) could meet RI because the intake amount of group above RI was as much as it compensate that of other groups

It is necessary for the group who intake vitamin B_{12} below RI to be provided with more vitamin B_{12}-rich food such as meat, milk, and egg through nutritional education and publicity. McLean *et al.* (28) reported that the high prevalence of vitamin B_{12} deficiency in many regions of the world is becoming recognized as a widespread public health problem and low plasma vitamin B_{12} concentrations in Kenya school children is improved by supplemental animal source food.

We calculated the correlation of the intake level between vitamin B_{12} and other nutrients whose database has been already set up by Rural Resources Development Institute (RRDI). Table 4 shows vitamin B_{12} intake has close correlation with vitamin B_2 intake and percent of energy from fat in comparison with other nutrients. It is assumed that foods of high vitamin B_{12} level also contain relatively high concentrations of vitamin B_2 and percent of energy from fat. People who like the vitamin B_{12}-rich food are likely to intake those nutrients, too ($p<0.001$) and they seem to be likely to intake animal foods.

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