Short Communication

The nutritional status of iron, folate, and vitamin B-12 of Buddhist vegetarians

Yujin Lee Dipl oec Troph and Michael Krawinkel PhD

Institute of Nutritional Sciences, Justus Liebig University, Giessen, Germany

Nutritional status of iron, folate, and vitamin B-12 in vegetarians were assessed and compared with those of non-vegetarians in Korea. The vegetarian subjects were 54 Buddhist nuns who ate no animal source food except for dairy products. The non-vegetarians were divided into two groups: 31 Catholic nuns and 31 female college students. Three-day dietary records were completed, and the blood samples were collected for analyzing a complete blood count, and serum levels of ferritin, folate, and vitamin B-12. There was no difference in hemoglobin among the diet groups. The serum ferritin and hematocrit levels of vegetarians did not differ from that of non-vegetarian students with a high intake of animal source food but low intake of vitamin C, and the levels were lower than that of non-vegetarian Catholic nuns with a modest consumption of animal source food and a high intake of vitamin C. The serum vitamin B-12 levels of all subjects except one vegetarian and the serum folate levels of all subjects except one non-vegetarian student fell within a normal range. In vegetarians, there was a positive correlation between the vitamin C intake and serum ferritin levels as well as between the laver intake and serum vitamin B-12 levels. In order to achieve an optimal iron status, both an adequate amount of iron intake and its bioavailability should be considered. Sufficient intake of vegetables and fruits was reflected in adequate serum folate status. Korean laver can be a good source of vitamin B-12 for vegetarians.

Key Words: iron, folate, vitamin B-12, Buddhist vegetarians, vegetarian diet

INTRODUCTION

Iron, folate, and vitamin B-12 are the crucial nutrients involved in active erythropoiesis, and their deficiencies result in decreased erythrocyte production, consequently leading to anemia.[1] Of these, iron deficiency is the most prevalent nutrient deficiency in both developing and industrialized countries, despite abundant iron supplies on earth.[2] Infant, children, and women of childbearing age, especially during pregnancy are mainly affected due to their high iron requirement.[3] Additionally, those who consume little animal source food or have a diet with low iron bioavailability can eventually develop iron deficiency.[4] The morphological characteristics of iron deficiency anemia are generally hypochromic and microcytic cells due to insufficient transport of hemoglobin to erythrocytes.[1] In contrast, the anemia caused by folate or vitamin B-12 deficiency is often macrocytic due to immature erythrocytes resulting from defective DNA synthesis.[5] A deficiency in either nutrient deactivates the methylation of homocysteine to methionine, which results in an elevated homocysteine level.[6] Moreover, a lack of vitamin B-12-dependent methionine synthase, which demethylates methyl tetrahydrofolate (THF), can lead to functional deficiency.[7] Because the hematological change in deficiency of either vitamin is indistinguishable and because these vitamins are metabolically interrelated, both should be assessed in order to differentiate between the two deficiencies.[8] In contrast to metabolic interaction between folate and vitamin B-12, their dietary sources are completely different. Whereas folate is mostly found in plants such as legumes, vegetables, and fruits,[9] vitamin B-12 is known to be predominantly present in animal tissue and generally absent in plants, with the exception of some seaweeds.[10-12] Therefore, vegetarians who consume less animal source food are considered a risk group for vitamin B-12 deficiency; although vitamin B-12 is seldom exhausted due to the enterohepatic circulation, and the clinical manifestation of vitamin B-12 deficiency rarely occurs, even without intake of vitamin B-12 for many years.[12]

Buddhist nuns and monks are the oldest vegetarian group in Korea. In accordance with Buddhist precepts, they have basically adhered to a vegan diet and have only occasionally consumed dairy products.[13,14] Therefore, they can be classified as lacto-vegetarians. The amount of consumed dairy products, however, is much lower than those of Western lacto-vegetarians; their intake of dairy products was comparable to "moderate" vegans.[13] With an increasing interest in vegetarian nutrition in Korea, the Buddhist vegetarian diet has been getting more attention. Nevertheless, the nutritional status of iron, folate, and vitamin B-12 in Buddhist vegetarians has not been investigated.

Corresponding Author: Dr Michael Krawinkel, Institute of Nutritional Sciences, Wilhelmstrasse 20, 35392 Giessen, Germany.
Tel: +49-641-99 39048; Fax: +49-641-99 39039
Email: michael.krawinkel@ernaehrung.uni-giessen.de
Manuscript received 30 June 2010. Initial review completed 20 September 2010. Revision accepted 1 November 2010.

Thus, we assessed the dietary intake as well as the hematological and biochemical status of iron, folate, and vitamin B-12 in Buddhist vegetarian nuns and compared them to those of non-vegetarian women in Korea. Additionally, correlations between dietary intake and blood parameters were determined.

MATERIALS AND METHODS

As described in more detail in a previous article,[13] the eligible vegetarian subjects were 54 Buddhist nuns between 21 and 44 years of age who had adhered to a vegetarian diet for more than 3 years. Except for the modest consumption of dairy products, they ate no animal source food such as meat, poultry, fish, or eggs. The non-vegetarians were divided into two groups. One consisted of 31 Catholic nuns between 21 and 41 years of age, whose dietary pattern is a plant-based diet with moderate consumption of animal source food. The other non-vegetarian group comprised 31 female college students between 18 and 31 years of age, who had a high intake of animal source food.

The participants included were those who 1) had regular menstrual cycles, 2) took neither dietary supplement nor medication for the last six months, 3) were apparently healthy, and had no physician-diagnosed disease (especially inflammatory disease).

This study was approved by the Ethics Committee of the Justus Liebig University in Giessen, Germany, and all participants were volunteers who had given written, informed consent to participate in the study.

Dietary assessment

All participants were asked to record food intake using household measures for three consecutive days before blood collection and were informed about portion size with food models and photographs.[15] In addition to the estimated amount of food intake, the main ingredients in meals were recorded, and we asked the nuns in charge of cooking to provide the vegetarian meal recipes. Based on this information, the standardized recipes[16] were modified and entered into the nutritional program. Can-Pro 2.0 (Computer Aided Nutritional Analysis Program version 2.0, The Korean Nutrition Society, 2002, Seoul) was used for analysis of dietary data.

Hematological and biochemical indicators

After overnight fasting, a venous blood sample was obtained from all participants. A complete blood cell count was determined by an automated hematology analyzer SE-9000 (TOA Medical Electronics, Kobe, Japan). Serum concentrations of ferritin, folate, and vitamin B-12 were measured by an Electrochemiluminescence Immunoassay (ECLIA), using commercial kits (Elecsys Ferritin, Folate, vitamin B-12 reagent kit, Roche Diagnostics, Mannheim, Germany) on the Elecsys 2010 analyzers (Roche Diagnostics, Mannheim, Germany).

WHO criteria for anemia were used for the reference value:[2] hemoglobin level <120 g/L and hematocrit <0.36. A serum ferritin level below 150 µg/mL is regarded to reflect depleted iron stores.[2] A mean corpuscular volume level below 80 fL is considered microcytosis, and over 100 fL is considered macrocytosis.[17] According to suggestions from WHO Technical Consultation on folate and vitamin B-12 deficiencies,[18] the cut-off value for defining deficiencies based on metabolic indicators was set at <10 nmol/mL for serum folate and at <150 pmol/mL for serum vitamin B-12.

Statistical analysis

Statistical analysis was performed using SPSS for Windows (version 17, Chicago, IL, USA). For group comparison, one-way ANOVA test was used, when the residuals were normally distributed (Kolmogorov-Smirnov test and Shapiro-Wilk test) and the variances equal (Levene's test). If a significant difference between the groups existed, a post hoc test (Scheffe) was followed for a multiple comparisons procedure that examined further differences between the groups. When the assumptions of ANOVA were not met, the Kruskal-Wallis test was performed. As a post hoc test, Mann-Whitney U test for pairwise comparison was used with Bonferroni's correction. By Pearson chi-square test, we determined whether the frequency of cases beyond the cut-off values were different among diet groups. Fisher's exact test was used when the expected value was less than 5. After the normal distribution tests, normally distributed data were assessed by Pearson correlation and non-normally distributed data by Spearman rank correlation coefficient. A p value <0.05 was considered statistically significant.

RESULTS

The dietary intake related to the status of iron, folate and vitamin B-12 in the study population is summarized in Table 1. Except for energy intake, the differences in nutrient intakes among three groups were noticeable. As expected, the vegetarian group had the highest intake of energy from plant sources, crude fiber, and folate and the lowest intake of energy from animal sources, animal source protein and iron. The non-vegetarian student group had the highest consumption of animal source foods; however, they had the lowest intake of total iron, folate, vitamin C, and crude fiber. The overall dietary pattern of the non-vegetarian Catholic nuns was similar to that of Buddhist vegetarians; however, they consumed, besides dairy products, modest amounts of animal source food such as meat, poultry, eggs, and seafood. Therefore their protein and iron intake from animal sources was higher than that of vegetarians but lower than that of non-vegetarian students. Additionally, they had the highest vitamin C intake due to high consumption of fruits during the research period.

The three major food groups that are sources of iron amounted to 77-78% of total iron intake in all diet groups. These are grains, vegetables including sea vegetables, and legumes in Buddhist vegetarians and non-vegetarian Catholic nuns. The non-vegetarian students had iron intake from not only grains and vegetables, but also from animal source foods, which accounted for 31% of total iron intake. The main dietary sources of folate in vegetarians were vegetables including sea vegetables and other groups, especially spices (78%). The non-vegetarian Catholic nuns had folate intake mostly from vegetables and legumes (75%). The non-vegetarian students consumed dietary folate, not only in vegetables, but also in

Table 1. Dietary intake of selected nutrients

	Buddhist vegetarians (n=54)	Omnivores		*p*-value
		Catholic nuns (n=31)	College students (n=31)	
Energy (kcal)	1756 (1371-2021)	1867 (1628- 2103)	1753 (1100-2285)	n.s‡
Protein (g)	52^{a} (13.3)	56^{a} (9.39)	66^{b} (12.5)	<0.001†
Total iron (mg)	14.1^{a} (10.9-17.3)	15.2^{a} (12.4-21.6)	10.0^{c} (8.9-11.9)	<0.001‡
Animal source iron (mg)	0.1^{a} (0.0-0.2)	1.0^{b} (0.8-1.3)	3.2^{c} (2.6-4.0)	<0.001‡
Plant source iron (mg)	14.1^{a} (10.7-17.1)	14.0^{a} (10.7-20.3)	6.8^{b} (5.2-8.3)	<0.001‡
Folate (µg)	340^{a} (296-430)	267^{b} (241-347)	177^{c} (152-219)	<0.001‡
Vitamin C (mg)	145^{a} (111-211)	197^{b} (141-286)	79.2^{c} (57.9-102.1)	<0.001‡
Crude fiber (g)	9.3^{a} (7.0-11.4)	7.6^{b} (6.6-8.9)	4.3^{c} (4.0-4.9)	<0.001‡
Energy from animal source food (%)	2.0^{a} (0.1-5.3)	5.0^{b} (3.7-6.8)	22.7^{c} (17.9-28.8)	<0.001‡
Animal source protein in total protein (%)	3.9^{a} (0.6-11.5)	21.7^{b} (17.4-27.3)	57.8^{c} (52.2-65.1)	<0.001‡
Animal source iron in total iron (%)	1.1^{a} (0.3-2.0)	6.5^{b} (4.1-9.4)	32.3^{c} (24.8-39.9)	<0.001‡
Laver (g)	1.3^{ab} (0.5-2.4)	1.3^{b} (1.0-2.0)	0.7^{a} (0.4-1.3)	0.005‡
Dairy product (g)	21.7 (5.0-67.1)	37.5 (32.0-115.0)	66.7 (0.0-156.7)	n.s‡

†One-way ANOVA, ‡Kruskal-Wallis test.
The data are presented as mean (SD) using one-way ANOVA and as median (1. -3. interquartile) using Kruskal-Wallis test. Means or medians with different superscript letter are significantly different between the groups after post hoc test (significant level: Scheffe 0.05, Bonferroni's correction 0.017). Means or medians with same superscript letter are not significantly different between the groups after post hoc test.
n.s not significant, *p*>0.05.

Table 2. Hematological and biochemical indicators of iron, folate, and vitamin B-12

	Buddhist vegetarians (n=54)	Omnivores		*p*-value
		Catholic nuns (n=31)	College students (n=31)	
Hemoglobin (g/L)	129 (90)	131 (95)	129 (93)	n.s†
Hematocrit	0.37^{a} (0.24)	0.39^{b} (0.24)	0.38^{a} (0.23)	<0.001†
Serum ferritin (µg/mL)	206^{a} (138-328)	373^{b} (243-574)	239^{ab} (165-471)	0.001‡
Serum folate (nmol/mL)	22.9^{a} (18.6-29.5)	19.7^{a} (17.9-24.9)	15.6^{b} (11.3-17.9)	< 0.001‡
Serum vitamin B_{12} (pmol/mL)	360^{a} (287-444)	527^{b} (422-638)	488^{b} (425-667)	< 0.001‡
Mean corpuscular volume (fL)	91.1^{a} (88.9-92.8)	94.4^{b} (92.5-96.9)	91.8^{a} (86.9-93.1)	< 0.001‡
Beyond the cutoff values (%)				
Hemoglobin <120 g/L (%)	22.2	6.5	12.9	n.s¶
HCT <0.36	29.6	6.5	19.4	0.039§
Serum ferritin <150 µg/mL (%)	27.8	6.5	19.4	n.s§
Serum folate <10 nmol/mL (%)	0	0	3.2	n.s¶
Serum vitamin B-12 <150 pmol/mL (%)	1.9	0	0	n.s¶
MCV <80 fL(%)	1.9	3.2	3.2	n.s¶
MCV >100 fL(%)	0	3.2	0	n.s¶

†One-way ANOVA, ‡Kruskal-Wallis test, §Pearson's chi-square test, ¶Fisher's exact test.
The data are presented as mean (SD) using one-way ANOVA and as median (1. -3. interquartile] using Kruskal-Wallis test. Means or medians with different superscript letter are significantly different between the groups after post hoc test (significant level: Scheffe 0.05, Bonferroni's correction 0.017). Means or medians with same superscript letter are not significantly different between the groups after post hoc test.
n.s not significant, *p*>0.05.

various food groups distributed relatively evenly. Of these, 11% of folate source was animal source food, and 15% was from other groups such as potatoes, nuts, and beverages, but little from spices.

Vitamin B-12 intake in our study was not directly calculated due to a lack of Korean national food data on vitamin B-12. Therefore, to assess the vitamin B-12 intake of vegetarians, the amount of dairy product and laver intake was considered (Table 1). The Buddhist vegetarians consumed almost exclusively plant food except for some dairy products. Thus, the dietary source of vitamin B-12 from animal foods in our vegetarians was exclusively dairy products. Additionally, Korean laver (*Porphyra tenera*), the most commonly consumed in Korea, is known to contain active vitamin B-12 and is regarded as a plant source of vitamin B-12.[19-21] There was no difference in laver intake between Buddhist vegetarians and non-vegetarian Catholic nuns, but the non-vegetarian students had the lowest intake of laver. The range of dairy product intake in all diet groups was very wide. Hence, even though the non- vegetarian students had the highest median value of dairy product intake, there was no significant difference in the intake of dairy products between groups, except between Buddhist vegetarians and non-vegetarian Catholic nuns.

The descriptive statistics of blood parameters and the frequency of cases beyond the cut-off values (%) are shown in Table 2. The means or medians of all blood parameters in all diet groups fell within the normal reference ranges. There was no significant difference in hemoglobin level between diet groups. 22.2% of vegetarians had a hemoglobin level between 109-119 g/L. The non-vegetarian Catholic nuns had the highest median of hematocrit and serum ferritin concentrations, whereas there was no difference in levels of hematocrit and serum ferritin between Buddhist vegetarians and non-vegetarian students.

The non-vegetarian students had the lowest and the vegetarian group the highest level of serum folate. None of vegetarians or Catholic nuns and only one student was folate deficient. The vegetarian group had the lowest serum vitamin B-12 level, but only one vegetarian had a level below 150 pmol/mL. There was no significant difference between the non-vegetarian groups and none of the non-vegetarians were deficient.

The non-vegetarian Catholic nuns had the highest mean corpuscular volume (MCV), whereas there was no significant difference between Buddhist vegetarians and omnivorous students. One subject from each group had microcytosis, and one Catholic nun had macrocytosis.

Using chi-square test and Fisher's exact test, we determined whether there are differences in the frequencies of cases beyond the cut-off values among the diet groups. Except for hematocrit (p=0.039), the different diets did not lead to statistical differences.

Except for a negative correlation between serum folate concentration and age in vegetarians (r=-0.303, p=0.026), blood variables were correlated with neither age, in all diet groups, nor duration of vegetarianism in vegetarians. Blood variables and nutrient intake are well correlated if the values of all subjects without subgroups are analyzed (Table3). Serum ferritin has a positive correlation with intake of animal protein (r=0.214, p=0.021) and animal source iron (r=0.213, p=0.022). Serum folate concentrations had a strong positive correlation with nutrient intake from plant sources such as folate (r=0.417, p<0.001), vitamin C (r=0.397, p<0.001), and plant protein (r=0.395, p<0.001) and a negative correlation with nutrient intake from animal sources such as animal protein (r=-0.520, p<0.001) and animal source iron (r=-0.517, p<0.001). In contrast, serum vitamin B_{12} was strongly positively correlated with animal protein (r=0.424, p<0.001) and animal source iron (r=0.414, p<0.001) and negatively correlated with intake of plant protein (r=-0.383, p<0.001) and folate intake (r=-0.222, p=0.016).

Serum ferritin concentration in vegetarians was positively correlated with vitamin C intake (r=0.315, p=0.020) and negatively correlated with energy intake (%) from total protein (r=0.274, p=0.045). In non- vegetarian Catholic nuns, serum ferritin concentration showed a strong negative correlation with nutrient intake from plant sources such as plant iron (r=-0.601, p<0.001), plant protein (r=-0.539, p=0.002), and carbohydrates (r=-0.603, p=0.000) and a positive correlation with energy intake from animal source food (r=0.407, p=0.023). Hemoglobin concentration in non- vegetarian Catholic nuns was negatively correlated with plant iron (r=-0,373, p=0.039) but not correlated with animal source iron. Conversely, in the student group, hemoglobin concentration was only positively correlated with animal source iron intake (r=0.369, p=0.041), and there was no correlation between serum ferritin concentration and nutrient intake.

No correlation between serum folate concentration and nutrient intake was found in either vegetarians or non-vegetarian Catholic nuns. However, serum folate in non-vegetarian students was positively associated with vitamin C intake (r=0.385, p=0.032) and negatively associated with energy intake (%) from animal fat (r=-0.403, p=0.025).

Only in vegetarians were serum vitamin B_{12} positively associated with laver intake (r=0.341, p=0.012). No asso-

Table 3. Correlation between blood variables and dietary intake

	All subjects	Buddhist vegetarians	Non- vegetarian	
			Catholic nuns	College students
Serum ferritin	Animal protein intake (+) Animal iron intake (+)	Vitamin C intake (+) Energy intake(%) from total protein (-)	Energy intake (%) from animal sources food (+) Plant iron intake (-) Plant protein intake (-) Carbohydrate intake (-)	
Hemoglobin			Plant iron intake (-)	Animal iron intake (+)
Serum folate	Folate intake (+) Vitamin C intake(+) Plant protein intake (+) Animal protein intake (-) Animal iron intake (-)			Vitamin C intake (+) Energy intake (%) from animal fat (-)
Serum vitamin B-12	Animal protein intake (+) Animal iron intake (+) Plant protein intake (-) Folate intake (-)	Laver intake (+) Energy intake (%) from total protein (+)		

+ positive correlation; - negative correlation.
p<0.05

ciation between serum vitamin B_{12} and the consumption of dairy product was found in all groups.

DISCUSSION

A diverse and balanced diet is considered best to provide all nutrients required for an adequate nutrient supply. All diets excluding a group of nutrients involve a risk of nutrient deficiencies. For vegetarians, iron and vitamin B-12 are nutrients likely to be insufficiently provided.

In order to achieve an optimal iron status, the amount of iron intake as well as iron bioavailability should be considered. A meatless diet has less of an effect on total iron intake; however, iron from this diet is generally less bioavailable than from an non-vegetarian diet.[22] In our study, the medians of total iron intake in Buddhist vegetarian nuns and non-vegetarian Catholic nuns were appropriate to Korean recommended daily intake (14 mg).[23] In contrast, even though non-vegetarian students had the highest intake of animal source iron, the total iron intake was much lower than the recommended daily intake. Iron bioavailability can be enhanced or inhibited by dietary factors, and the iron absorption rate varies more than tenfold depending on meal composition.[24] Vitamin C is known as the strongest enhancer of iron absorption and can improve the iron bioavailability in natural and fortified foods against inhibitors such as phytate, polyphenols, calcium, and protein in dairy products.[24-26] Thus, a high vitamin C intake by vegetarians can compensate for the low iron bioavailability in a vegetarian diet. In fact, vitamin C intake of our vegetarians was positively correlated with serum ferritin concentration. Additionally, even though the non-vegetarian students with the lowest vitamin C intake consumed the highest amount of animal source food, their serum ferritin concentration did not differ from that of Buddhist vegetarians and was even slightly lower than that of non-vegetarian Catholic nuns with a modest consumption of animal source food and a high intake of vitamin C simultaneously. Intake of animal source iron is associated with a high iron bioavailability; however, that alone does not seem to lead to an optimal iron status.[27]

Serum ferritin is the most sensitive indicator of depleted iron stores. The serum ferritin level of our vegetarians was comparable to that of other vegetarian women and higher than that of young vegan German women, whereas hemoglobin concentration was very similar.[28,29] It is a common finding that there is no significant difference in hemoglobin or serum ferritin concentrations between vegetarian and non- vegetarian women.[27,29-31]

As in other vegetarians,[28,31,32] grains, vegetables, legumes, and fruits were the main dietary sources of iron in the Buddhist vegetarians. Additionally, unlike Western vegetarians, they had an iron intake from sea vegetables (5%). Because sea vegetables contain no phytate, they are considered a good source of iron.[36] Besides, they contain vitamins, minerals, and trace elements, especially iodine which is also a nutrient of concern in vegetarian diets.[33-35] In contrast, breakfast cereals playing an important role as iron source in Western vegetarians,[28,32] were not consumed.

As ferritin is one of the acute-phase proteins that increases in the presence of infection or inflammation, the cut-off value for iron deficiency below 150 µg/mL may be not valid under inflammatory conditions.[36] Although we did not determine acute-phase proteins, our subjects were asked for their disease history instead. In the analysis, the subjects free of acute or chronic diseases and not taking any medication were included.

The median folate intake of all diet groups did not reach the amount of Korean recommended daily intake (400 µg).[23] Nevertheless, the serum folate levels of all 54 Buddhist vegetarians (range 14.30-45.3 nmol/mL), 31 non-vegetarian Catholic nuns (range 10.4-32.9 nmol/mL), and 29 non-vegetarian students except one (range 8.5-21.7 nmol/mL) fell within the normal level (>10 nmol/mL). The serum folate level of our vegetarians was lower than that of vegetarians in other studies.[29,37] As serum folate concentration is determined by recent dietary intake,[8] the lower levels may reflect that the blood samples were collected in winter and early spring when dietary folate sources were limited. Another explanation could be that our subjects did not consume folate fortified foods and therefore have lower folate intake compared to people eating fortified food. Furthermore, a serum folate level >45.3 nmol/mL may not be desirable, especially combined with low serum vitamin B-12, because it could mask vitamin B-12 deficiency ("folate trap").[7]

The dietary sources of folate were mainly vegetables, grains, and legumes. As with dietary iron, sea vegetables were a good folate source for Buddhist vegetarians as well as the non-vegetarian, contributing 10-18% of total folate intake.

Main dietary sources of vitamin B-12 for vegetarians are milk and eggs, although dairy products contain very small amount of vitamin B-12 and bioavailability from eggs is low.[10,38] For Western vegetarians, especially vegans fortified foods increase vitamin B-12 supply.[39] As potential dietary sources of vitamin B-12 for Asian vegetarians, plant foods such as fermented soy products and seaweeds were investigated in many studies.[10-12,19,20] Most of them detected either exclusively vitamin B-12 analogues or traces of *true* vitamin B-12. Exceptionally, substantial amounts of vitamin B-12 were found in purple (*Porphyra sp*.) and green (*Enteromorpha sp.*) algae.[11,19-21,40] Vitamin B-12 content in laver varies depending on analysis method and species. Korean laver (*Porphyra tenera*) is the most commonly consumed seaweed species in Korea, as well as worldwide.[41] Kwak *et al*. found that 100 g of Korean laver products (*P. tenera*) contained 66.8 µg of vitamin B-12.[20] Miyamoto *et al*. reported a vitamin B-12 content in Korean dried laver (*P. tenera*) of 134 µg and in seasoned and toasted laver 51.7 µg.[19] Furthermore, no vitamin B-12 analogues were found in this laver. Similarly, raw laver (*P. tenera*) in the studies of Yamada *et al.* had a high proportion of *true* vitamin B-12 73-100%, whereas dried laver had only 35% *true* vitamin B-12.[11,21]

The bioavailability of vitamin B-12 in laver for human is still controversial. A Dutch study on macrobiotic children found laver (*P. tenera*), spirulina, and fermented plant foods not reliable as sources of vitamin B-12.[42] After consuming vitamin B-12 from only these plant foods for 5 months, MCV in five vegan children was even higher, despite increasing plasma vitamin B-12 levels. Methylmalonic acid is considered as the most important functional indicator for vitamin B-12, because this vitamin is necessary for the conversion of methylmalonyl CoA to succinyl CoA. In vitamin B-12 deficiency, methylmalonic acid concentration is high in serum and urine.[43] A study on the bioavailability of vitamin B-12 in laver using methylmalonic acid showed that raw laver was more reliable dietary source of vitamin B-12 for vegetarians than dried laver.[21] However in another study, six Japanese vegan children who daily consumed 2-4 g of dried laver had neither vitamin B-12 deficiency in blood nor neurological disturbances.[44]

In our study, most of the subjects consumed dried laver; however, only the vegetarian group showed a positive correlation between laver intake and serum vitamin B-12 levels. Buddhist vegetarians had a high serum vitamin B-12 concentration compared to other vegetarians.[29,37] On the basis of its high content of *true* vitamin B-12, Korean laver can be recommended for vegetarians as a dietary source of vitamin B-12.[10,19-21] Nevertheless, more studies on its bioavailability from Korean laver in vegetarians are desirable.

In contrast to other studies,[45-47] dairy intake had no effect on vitamin B-12 status here. One reason could be the low intake of dairy products. Korea is one of the countries with low milk consumption.[48] The National Health and Nutrition Survey reported that the average consumption of milk products of the female population was 70.6 g per day.[49]

In this study, the nutritional status of iron, folate, and vitamin B-12 in Buddhist vegetarians were appropriate. Although they had very low intake of animal source food, their prevalence of deficiencies of these nutrients did not differ from that of non-vegetarian who consumed larger amounts of animal source foods. High intake of vegetables and fruits was well reflected in the adequate serum folate status. In contrast to Western vegetarians, fortified foods and milk are not relevant dietary sources of iron, folate and vitamin B-12 for Korean vegetarians. Instead, sea vegetables contribute more to the supply of these nutrients. Therefore, dietary guidelines for Korean vegetarians based on Korean food culture should be developed.

ACKNOWLEDGEMENTS

We thank the department of food and nutrition, the department of physiology and the department of clinical pathology (College of Medicine) at Yeungnam University in South Korea.

AUTHOR DISCLOSURES

The Authors declare that they have no conflict of interest.

REFERENCES

1. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr. 2004; 24:105-31.
2. World Health Organization, United Nations Children's Fund, United Nations University. Iron Deficiency Anaemia; Assessment, Prevention and Control; A guide for programme managers. World Health Organization; 2001.
3. World Health Organization. Worldwide prevalence of anaemia 1993-2005: WHO Global Database on Anaemia. Geneva: WHO Press; 2008.
4. Denic S, Agarwal MM. Nutritional iron deficiency: an evolutionary perspective. Nutrition. 2007;23:603-14.
5. Metz J. A high prevalence of biochemical evidence of vitamin B12 or folate deficiency does not translate into a comparable prevalence of anemia. Food Nutr Bull. 2008;29:74-85.
6. Scott JM. Folate and vitamin B12. Proc Nutr Soc. 1999;58: 441-8.
7. Selhub J, Morris MS, Jacques PF, Rosenberg IH. Folate-vitamin B-12 interaction in relation to cognitive impairment, anemia, and biochemical indicators of vitamin B-12 deficiency. Am J Clin Nutr. 2009;89:702-6.
8. Green R. Indicators for assessing folate and vitamin B12 status and for monitoring the efficacy of intervention strategies. Food Nutr Bull. 2008;29:52-63.
9. Suitor CW, Bailey LB. Dietary folate equivalents: interpretation and application. J Am Diet Assoc. 2000;100:88-94.
10. Watanabe F. Vitamin B12 sources and bioavailability. Exp Biol Med. 2007;232:1266-74.
11. Yamada S, Shibata Y, Takayama M, Narita Y, Sugawara K, Fukuda M. Content and characteristics of vitamin B12 in some seaweeds. J Nutr Sci Vitaminol. 1996;42:497-505.
12. Herbert V. Vitamin B-12: plant sources, requirements, and assay. Am J Clin Nutr. 1988;48:852-8.
13. Lee Y, Krawinkel M. Body composition and nutrient intake of Buddhist vegetarians. Asia Pac J Clin Nutr. 2009;18:265-71.
14. Kim JA, Lee SY. Evaluation of served menu and management of foodservice in Korean Buddhist temples. J East Asian Soc Dietary Life. 2006;16:215-25.
15. Korean Dietetic Association. Eye measurement of food with photograph. Seoul: Korean Dietetic Association; 2002.
16. Ministry of Health and Welfare. Report on project for database construction of nutritional component analysis material by food groups. Gwacheon: Ministry of Health and Welfare; 2000.
17. Tefferi A. Anemia in adults: a contemporary approach to diagnosis. Mayo Clin Proc. 2003;78:1274-80.
18. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. Food Nutr Bull. 2008;29:238-44.
19. Miyamoto E, Yabuta Y, Kwak CS, Enomoto T, Watanabe F. Characterization of vitamin B12 compounds from Korean purple laver (Porphyra sp.) products. J Agric Food Chem. 2009;57:2793-6.
20. Kwak CS, Hwang JY, Watanabe F, Park SC. Vitamin B12 contents in some Korean fermented foods and edible seaweeds. Korean J Nutr. 2008;41:439-47.
21. Yamada K, Yamada Y, Fukuda M, Yamada S. Bioavailability of dried asakusanori (porphyra tenera) as a source of Cobalamin (Vitamin B12). Int J Vitam Nutr Res. 1999;69:412-8.
22. Hunt JR. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am J Clin Nutr. 2003;78:633-9.
23. The Korean Nutrition Society. Dietary Reference Intakes For Koreans 8th Revision. Seoul: The Korean Nutrition Society; 2005.
24. Hallberg L, Hulthen L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. Am J Clin Nutr. 2000;71:1147-60.
25. Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr. 2010;91(Suppl):1461S-7S.
26. Hunt JR. Dietary and physiological factors that affect the absorption and bioavailability of iron. Int J Vitam Nutr Res. 2005;75:375-84.
27. Harvey LJ, Armah CN, Dainty JR, Foxall RJ, John LD, Langford NJ, Fairweather-Tait SJ. Impact of menstrual blood loss and diet on iron deficiency among women in the UK. Br J Nutr. 2005;94:557-64.
28. Waldmann A, Koschizke JW, Leitzmann C, Hahn A. Dietary iron intake and iron status of German female vegans:

results of the German vegan study. Ann Nutr Metab. 2004;48:103-8.
29. Obeid R, Geisel J, Schorr H, Hubner U, Herrmann W. The impact of vegetarianism on some haematological parameters. Eur J Haematol. 2002;69:275-9.
30. Haddad E, Berk LS, Kettering J, Hubbard R, Peters W. Dietary intake and biochemical, hematologic, and immune status of vegans compared with nonvegetarians. Am J Clin Nutr. 1999;70:586-93.
31. Shaw NS, Chin CJ, Pan WH. A vegetarian diet rich in soybean products compromises iron status in young students. J Nutr. 1995;125:212-19.
32. Ball MJ, Bartlett MA. Dietary intake and iron status of Australian vegetarian women. Am J Clin Nutr. 1999;70:353-8.
33. Garcia-Casal MN, Pereira AC, Leets I, Ramirez J, Quiroga MF. High iron content and bioavailability in humans from four species of marine algae. J Nutr. 2007;137:2691-5.
34. Tokudome S, Tokudome Y, Goto C, Suzuki S, Moore MA. Seaweed as a beneficial iodine food source. Asian Pac J Cancer Prev. 2004;5:89.
35. Ruperez P. Mineral content of edible marine seaweeds. Food Chem. 2002;79:23-6.
36. World Health Organization, Centers for Disease Control and Prevention. Assessing the iron status of populations. Geneva: World Health Organization; 2007.
37. Herrmann W, Obeid R, Schorr H, Geisel J. Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk. Clin Chem Lab Med. 2003;41:1478-88.
38. Chanarin I, Metz J. Diagnosis of cobalamin deficiency: the old and the new. Br J Haematol. 1997;97:695-700.
39. Craig WJ, Mangels AR. Position of the American Dietetic Association: vegetarian diets. J Am Diet Assoc. 2009;109:1266-82.
40. Watanabe F, Takenaka S, Kittaka-Katsura H, Ebara S, Miyamoto E. Characterization and bioavailability of vitamin B12-compounds from edible algae. J Nutr Sci Vitaminol. 2002;48:325-31.
41. McHugh DJ. A guide to the seaweed industry: FAO FISHERIES TECHNICAL PAPER 441. Rome: Food and Agriculture Organization of the United Nations; 2003.
42. Dagnelie PC, van Staveren WA, van den BH. Vitamin B-12 from algae appears not to be bioavailable. Am J Clin Nutr. 1991;53:695-7.
43. Selhub J, Jacques PF, Dallal G, Choumenkovitch S, Rogers G. The use of blood concentrations of vitamins and their respective functional indicators to define folate and vitamin B12 status. Food Nutr Bull. 2008;29:67-73.
44. Suzuki H. Serum vitamin B12 levels in young vegans who eat brown rice. J Nutr Sci Vitaminol. 1995;41:587-94.
45. Tucker KL, Rich S, Rosenberg I, Jacques P, Dallal G, Wilson PW, Selhub J. Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring study. Am J Clin Nutr. 2000;71:514-22.
46. Koebnick C, Hoffmann I, Dagnelie PC, Heins UA, Wickramasinghe SN, Ratnayaka ID, Gruendel S, Lindemans J, Leitzmann C. Long-term ovo-lacto vegetarian diet impairs vitamin B-12 status in pregnant women. J Nutr. 2004;134:3319-26.
47. Vogiatzoglou A, Smith AD, Nurk E, Berstad P, Drevon CA, Ueland PM, Vollset SE, Tell GS, Refsum H. Dietary sources of vitamin B-12 and their association with plasma vitamin B-12 concentrations in the general population: the Hordaland Homocysteine Study. Am J Clin Nutr. 2009;89:1078-87.
48. Speedy AW. Global production and consumption of animal source foods. J Nutr. 2003;133:4048-53.
49. Korea Health Industry Development Institute, Ministry of Health and Welfare. Report on 2001 National Health and Nutrition Survey. 2003.

Short Communication

The nutritional status of iron, folate, and vitamin B-12 of Buddhist vegetarians

Yujin Lee Dipl oec Troph and Michael Krawinkel PhD

Institute of Nutritional Sciences, Justus Liebig University, Giessen, Germany

佛教素食者之鐵、葉酸及維生素 B-12 之營養狀況

本研究比較韓國素食者與非素食者之鐵、葉酸及維生素 B-12 之營養狀況。研究對象之素食者共 54 位比丘尼，其飲食除了乳製品以外，其它動物性來源食品皆不攝取。在非素食者部分分成兩組，分別為 31 位修女及 31 位女性大學生。研究方法：使用三天飲食記錄，同時收集血液樣本，分析完整血液指標與血清鐵蛋白、葉酸及維生素 B-12 濃度。結果發現不同飲食組別，其血紅素濃度沒有顯著差異。另外素食者血清鐵蛋白與血比容百分比，與有較高動物食品攝取量，及較低維生素 C 攝取量之非素食的大學生組相比，沒有顯著差異；但是與適量動物性食品攝取，及較高維生素 C 攝取之非素食修女組相比，則有較低的情況。除了一位素食者與一位非素食的大學生以外，所有研究對象的血清維生素 B-12 濃度與葉酸濃度，都在正常的範圍之內。在素食者發現，維生素 C 攝取量與血清鐵蛋白濃度存在正相關，另外紫菜攝取量與血清維生素 B-12 濃度也存在正相關。為了達到適當的鐵營養狀況，必須同時考慮鐵的攝取量及其生物利用率。適量血清葉酸濃度反映出受試者蔬果攝取充足。最後發現韓國的紫菜是素食者維生素 B-12 很好的來源。

關鍵字：鐵、葉酸、維生素 B-12、佛教素食者、 素食飲食