韓國營養學會誌 27(9) : 930~939, 1994
Korean J Nutrition 27(9) : 930~939, 1994

Maternal Vitamin B-6 Intake and Pyridoxine Status of Korean Newborns at Parturition*

Chang, Namsoo · Kim, Kyunghee** · Chung, Hyekyung · Song, Eunseung

Department of Food and Nutrition, Hoseo University, Asan-kun, Chungnam, Korea
*Department of Pediatrics,** Ewha Womans University Medical School, Seoul, Korea*

ABSTRACT

Vitamin B-6 nutrition has been shown to be inadequate in many population groups including pregnant and lactating women, and in infants. Vitamin B-6 intake was measured in 98 pregnant mothers and a total of 172 cord blood samples of their and other new born infants were analyzed for erythrocyte alanine aminotransferase(EALAT) activities with or without the addition of pyridoxal-5-phosphate to assess vitamin B-6 status of the infants. The average daily vitamin B-6 intake of the pregnant mothers was 1.79mg± 0.88(81.4% of the Recommended Dietary Allowances ; RDA) and vitamin B-6 to protein intake ratio was 0.017mg vitamin B-6/g protein. Thirty-eight percent of the pregnant women consumed diets which provided less than the RDA for vitamin B-6 during pregnancy. Seventy-two percent of the dietary pyridoxine intake was provided by the plant food source whose bioavailability was reported to be lower when compared to that of the animal food. The average activity coefficient(AC) values of the cord blood EALAT was 1.41± 0.11, and 32% of the blood samples had EALATAC values greater than 1.25, suggesting that vitamin B-6 status of the newborns might be less than adequate.

KEY WORDS : vitamin B-6 intake · vitamin B-6 status · pyridoxine · pregnancy · newborn infants · erythrocyte alanine aminotransferase activity coefficients.

Introduction

Vitamin B-6 nutritional inadequacy has been documented among many different population groups including pregnant women and new-born infants in various nations[1-5]. Vitamin B-6 intake has been shown to be positively correlated with the income and education level in the US[6], and several studies have documented positive correlations between maternal vitamin B-6 intake, maternal and cord blood levels of vitamin B-6. In underdeveloped countries maternal and cord blood has been found to be deficient in vitamin B-6[7][8].

Vitamin B-6 requirement is increased during pregnancy and inadequate vitamin B-6 nutrition affects the outcome of pregnancy and the birth status of the newborn infants[9][10] Vitamin B-6 influences the metabolism of neurotransmitters, sphingolipids in the central nervous system[11][12]. Animals raised on a vitamin B-6 deficient diet have been shown to cause morphological changes

Accepted : Octoher 21, 1994
*본 연구는 한국과학재단 기초연구지원과제로 수행되었던 연구의 일부임.

in cerebellum and decreased synaptic synthesis in the cerebral cortex long before any clinical vitamin B-6 deficiency symptoms appear[13][14].

Koreans consume foods mainly of plant origin which are known to contain vitamin B-6 in glycosylated form with relatively poor bioavailability. Since good sources of vitamin B-6 are limited to animal foods that are more expensive, it is likely that the vitamin B-6 intake of Koreans, and especially of Korean pregnant women might be marginally insufficient.

The purposes of this study were :

1) to ascertain vitamin B-6 intake data among Korean pregnant women.

2) to identify food sources contributing to the vitamin B-6 intake.

3) to examine whether maternal vitamin B-6 intake has influences on the vitamin status of the newborn infants at birth.

Materials and Methods

1. Study Subjects

We recruited 98 mothers who were admitted to the maternity ward of Ewha Womans University Hospital for delivery. They were apparently healthy and seemed to be free of any diseases other than having their babies delivered.

2. Demographic and Socioeconomic Data

A questionnaire was formulated to collect demographic and socioeconomic data from the pregnant participants by the trained interviewers. Data on height and weight of the mothers and of the newborn infants, household income, food expenditure, and education level were obtained

3. Food and Nutrient Intake Data

Food and nutrient intake data were collected from the pregnant mothers via a combined food frequency questionnaire and 24-hour recall method by the trained interviewers. The food frequency questionnaire was designed specifically to identify food sources and the amount of vitamin B-6 intake. Any changes in food intake behavior and food preferences occurred during the prenatal period were also noted.

Since vitamin B-6 values of Korean foods are not yet available, we used USDA Handbook 8 and Food Values of Portions Commonly Used to determine vitamin B-6 contents of foods that were consumed by the study participants[15][16]. Ewha software program was used to estimate energy and other nutrient intake.

4. Erythrocyte Alanine Aminotransferase Activity Coefficients

One hundred seventy two cord blood samples were centrifuged, plasma and buffy coats removed, and washed with physiological saline solution twice and erythrocyte samples were prepared immediately and stored frozen at 20℃ until the assay was perfomed.

Cord blood samples were analyzed for erythrocyte alanine aminotransferase(EALAT) activity spectrophotometrically with or without exogenous pyridoxal-5-phosphate to assess vitamin B-6 status [17]. EALAT activity coefficient was calculated from the following formula.

$$\text{EALAT AC} = \frac{\text{EALAT}(+\text{PLP}) - \text{EALAT}(\text{no PLP})}{\text{EALAT}(\text{no PLP})}$$

Hemoglobin concentations of the erythrocyte hemolysates were determined colorimetrically by the Drabkin method[18].

5. Statistical Analysis

Statistical Analytical System(SAS) package was used for the analyses of the data[19]. A stepwise multiple regression analysis was performed to

identify any variables which might influenced vitamin B-6 status of the newborn infants. To analyze statistical significances of the mean values from the different groups, a T-test was performed. A quartile analysis was performed to determine whether maternal vitamin B-6 intake influenced on the vitamin status of the cord blood of the newborn infants. Maternal vitamin B-6 intake levels were divided into 4 quartiles and cord blood EALATAC values of the two groups whose maternal vitamin B-6 intake were ranked at the top 25% and bottom 25% were compared.

Results and Discussion

1. Physical characteristics of the subjects

Physical characteristics of the study subjects are shown in the Table 1. The average age of the pregnant mothers was 28.9 years. When divided into two age groups(<30 years and >30 years), there was no significant differences in prepregnancy weight, weight gain during pregnancy, and body mass index among the groups. However, maternal height of the age group younger than 30 years was significantly greater by 2.2cm than the other.

2. Nutrient Intake of Pregnant Women

Pregnant women of the present study consumed 2,635 Calories and 100.9g of protein which are 112 percents of the Korean RDAs for energy and protein(2,350Cal and 90g) during pregnancy, and their energy and protein intake seemed to be adequate(Table 2). Percent energy contributions from protein, fat, and carbohydrates were 14.7, 21.8, and 63.5, respectively, which was close to what is being currently recommended by the Korean nutrition scientists(15 : 20 : 65). They consumed 923mg of calcium and were almost able to meet the RDA for pregnancy(1,000mg/day). There is a big regional difference in the amount of calcium intake among pregnant women in Korea because of the high cost and other reasons to avoid calcium-rich milk and milk products[20]. Pregnant women in the rural areas consume 400~500mg in average, which is considerably lower than those in the urban areas(800~900mg). Since our study subjects were from the middle to upper-middle socioeconomic classes with high income and education levels, their calcium intake was relatively high.

3. Vitamin B-6 Intake

The average daily vitamin B-6 intake of the subject was 1.79mg(Table 2). When compared to the US RDA values[21](since no Korean RDAs for vitamin B-6 has yet been established), our subjects consumed 81.4 % of the recommended level of vitamin B-6(2.2mg). Thirty eight percent of the subjects(37 out of 98) consumed less than the RDA for vitamin B-6 during pregnancy. Vitamin B-6 intake of our subjects was comparable to that reported by Newman and Norcross who estimated vitamin B-6 intake of the low-income

Table 1. Physical characteristics of the subjects

Measurements	Age of the Mothers	
	20~29 yrs(n=48)	>30 yrs(n=48)
Height(cm)	158.91± 0.78	156.73± 0.69*1)
Prepregnancy Wt(kg)	52.52± 1.05	52.49± 1.58
Wt at parturition(kg)	64.24± 1.04	63.32± 1.56
Body Mass Index(kg/m^2)	21.29± 0.54	20.61± 0.36

1) Means± SE

*Values are significantly different from each other at $P<0.05$.

Table 2. Nutrient intake of pregnant women

Nutrients	Amount[1)]	% Korean RDA[2)]
Energy(Cal)	2635.54± 130.97	112.2
Protein(g)	100.94± 6.46	112.2
Fat(g)	66.47± 6.93	
Carbohydrate(g)	435.09± 31.06	
Calcium(mg)	922.98± 52.81	92.3
Phosphorus(mg)	1534.55± 88.38	
Iron(mg)	28.12± 1.12	140.6
Vitamin B-1(mg)	2.07± 0.14	147.9
Vitamin B-2(mg)	2.41± 0.13	160.7
Vitamin B-6(mg)	1.79± 0.88	81.4[3)]
Niacin(mg)	24.83± 1.63	160.0
mg B-6/g protein	0.017	
% energy pro : fat : CHO	14.7 : 21.8 : 63.5	

1) Means SE

2) Korean RDA for pregnant women : energy(2,350 Cal), protein(90g), calcium(1,000mg), iron(20mg), vitamin B-1(1.4mg), vitamin B-2(1.5mg), niacin(15mg).

3) US RDA for vitamin B-6 during pregnancy : 2.2mg/day

Southeast Asian pregnant women in the US[22)]. Reynolds, et al. reported that non-lactating pregnant women in the US consumed 1.14mg of vitamin B-6, and only 6% of the women met RDA for vitamin B-6 during pregnancy[23)].

Vitamin B-6 nutrition is related to protein nutrition as vitamin B-6 is involved in many facets of amino acid metabolism. It has been shown by several researchers that protein intake influences the requirement of vitamin B-6[24-26)]. When vitamin B-6 intake was divided by the protein intake, our study subjects consumed 0.017mg vitamin B-6/g protein. Kant and Block reported that the US pregnant women consume 0.018~0.020mg vitamin B-6/g protein[6)]. It has been suggested an intake of 0.016mg vitamin B-6/g of protein to be an optimum one for non-pregnant adult women [21)]. However, such guideline has not yet been established for pregnant women.

The extra protein allowances for pregnancy should be accompanied by additional vitamin B-6. Vitamin B-6 status has often been reported to be inadequate during pregnancy as shown in the lower levels of PLP or B-6 vitamers and decreased PLP-dependent enzyme activities in the plasma or erythrocytes compared to non-pregnant women [27-30)]. Food and Nutrition Board recommends an additional 0.6mg of vitamin B-6 to the pregnant women to accommodate increased protein intake and to maintain an adequate vitamin B-6 nutritional status during pregnancy[21)].

Several investigators have reported that vitamin B-6 requirement during pregnancy should be increased further than the current RDA for pregnant women, and as much as 4-10mg per day may be required for some pregnant women[27-30)]. Inadequate vitamin B-6 nutrition during pregnancy has been associated with poor pregnancy outcome as shown in the increased frequencies of low-birth weight infants and lower Apgar scores[1)10)32)]. Kondo et al[33)]. reported that even though vitamin B-6 intake during the gestational period was more than adequate, vitamin B-6 status of the rat pups was poor and more vitamin B-6 than the recom-

mended level had to be provided to the lactating rats to ensure proper physical and neuromuscular development of the offsprings.

However, there are some reports suggesting that the lowered vitamin B-6 status may simply represent normal, relatively benign changes in pregnancy. Sloger & Reynolds[34)35)] found that vitamin B-6 supplementation during pregnancy and lactation did not improve on the vitamin B-6 status of the rat pups. Furth-Walker, et al[36)]. found that during pregnancy plasma PLP is decreased by 50%, while PLP in erythrocyte and whole blood increased suggested that metabolism or utilization of vitamin B-6 is altered during pregnancy.

4. Food Groups Contributing to the Vitamin B-6 Intake

Foods that were consumed by the participants were analyzed by the food groups. As shown in the Figure 1, vegetables and fruits contributed vitamin B-6 the most(40.7%), followed by grains and cereals(28.1%), meat, fish and poultry(17.1%), milk and milk products(8.5%), legumes(2.8%), eggs(1.8%), and seaweeds(0.2%). Plant food provided 71.8%, more than 2/3 of the total vitamin B-6 intake.

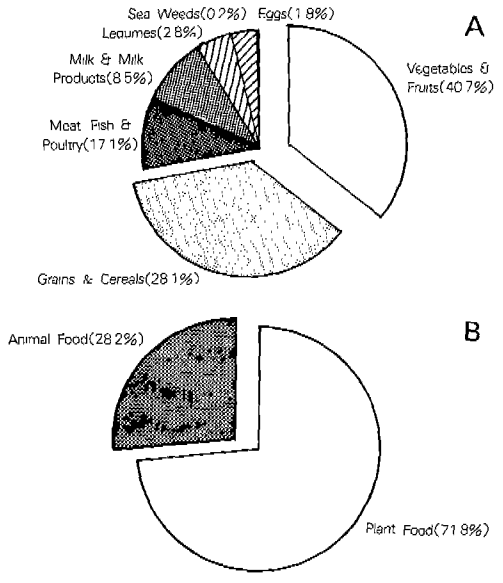

Fig. 1. A. Contributions made by Food Groups to Vitamin B-6 Intake.
B. Contributions made by Animal and Plant Food to Vitamin B-6 Intake

The richest sources of vitamin B-6 are chicken, fish, organ meats, and pork. Whole grains and legumes are also good sources of vitamin B-6. Vitamin B-6 occurs mainly in animal foods which are relatively expensive and Kant and Block[6)] reported that vitamin B-6 intake was positively correlated with income and education levels in the US.

However, milled grains such as polished rice, milled wheats, legumes, vegetables and fruits are the poor sources of vitamin B-6 because B-6 occurs in bound forms in nature. Vitamin B-6 in plant foods is bound to glucose, and its bioavailabilities have been shown to be questionable. Glycosylated vitamin B-6 cannot be utilized by human beings, although it is absorbed well[37-39)].

5. Usuage of Vitamin B-6 Containing Nutritional Supplements

Twenty women(22.4%) of the study subjects were taking commercially available nutritional supplements containing pyridoxine · HCl. Table 3 shows the contents of vitamin B-6 of the commercially available prenatal nutritional supplements. These supplements contain 0.08mg~20.0 mg of pyridoxine · HCl per tablet, our subjects consumed Materna(the most frequently used one) and Hemo-Contin which contain 4.86mg and 5.0mg of pyridoxine HCl(3.0mg and 3.08mg of pyridoxine), respectively. There was no differences in vitamin B-6 intake from food among the women regardless of their usuage of the supplements(Table 4). Notably, several participants were taking three tablets of the supplements daily, which might result in excessive intake of some

of the fat-soluble vitamins and minerals.

6. Vitamin B-6 status of the Newborns at Birth

Vitamin B-6 status of the newborns at birth was assessed by measuring erythrocyte alanine aminotransferase activities with or without the additional of exogenerous PLP. The average EALAT activity coefficient of 171 cord blood samples was 1.41~0.11. When the conservative criterion for vitamin B-6 inadequacy suggested by Sauberlich, et al[17](EALAT AC greater than 1.25). is used, 32% (55 out of 172) of the cord blood samples were shown inadequate in vitamin B-6 status. Among these samples, 9.9% had EALAT AC values greater than 2.00(Table 5).

Erythrocyte alanine transaminase activity coefficient values of the cord blood were significantly higher for the group whose mothers' vitamin B-6 intake was in the lowest quartile(Table 6). In the previous report we documented that energy, protein, iron, vitamin B-2 intake among other things also positively influenced the vitamin B-6 status of the newborns at birth[40].

Along with the other water-soluble B vitamins, vitamin B-6 is transported from the the maternal circulation to the fetus actively, securing a good supply of vitamin B-6 to the fetus[27]. However, when maternal vitamin B-6 nutrition is inadequate, fetus might not be able to do so. Roepke and Kirksey reported that there is a positive correlation between maternal vitamin B-6 intake and the erythrocyte PLP concentrations of the cord blood[29]. Ramsey et al[7]. measured EALAT AC of the maternal and cord blood from the Kenyan people of the low socioeconomic status. They found that 35% of the maternal blood and 15% of the cord blood sample were deficient in vitamin B-6[8]. They also reported that the activity of diamine oxidase, another PLP-dependent enzyme,

Table 3. Pyridoxine content of the commercial nutritional supplements

Commercial Supplements	Amount of pyridoxineo HCl* mg per tablet
Materna	4.86
Hivinal S	10.0
Vinapollo A	2.0
Supradyn	10.0
Grant	7.29
Centrum	3.65
Hemo Contin	5.0
Hanbangyeosungmo	0.08
Vitomin	20.0
Poltespan	5.0

*61.73% of pyridoxine HCl is free pyridoxine.

Table 4. Supplement usuage and vitamin B-6 intake from food

	Vitamin B-6 Intake from Food (mg/day)
Supplemented	1.74± 0.84[1] (76)
Unsupplemented	1.80± 0.90 (22)

1) Means± SE
2) Number of subjects
Values are not significantly different from each other.

Table 5. Distribution of cord blood EALAT AC values

EALAT AC Values[1]	Number of Subjects(%)
<1.25	117(68.0%)
1.25~2.00	38(22.1%)
>2.00	17(9.9%)

1) EALAT activity coefficient was calculated from the following formula.

$$\text{EALAT AC} = \frac{\text{EALAT}(+\text{PLP}) - \text{EALAT}(\text{no PLP})}{\text{EALAT (no PLP)}}$$

Table 6. Maternal vitamin B-6 intake and cord blood EALATAC

	EALATAC
Vitamin B-6 Q_1	1.27± 0.23*
Intake Q_3	0.71± 0.16

Q_1 : maternal vitamin B-6 intake bottom 25%
Q_3 : maternal vitamin B-6 intake top 25%
EALATAC : erythrocyte alanine aminotransferase
*significant at $p<0.05$

was also low in these subjects. Kaminetzky et al[30]. reported that birth weight of the infants was significantly low when maternal vitamin B-6 levels were low. Schuster et al[10]. reported that the Apgar scores at 1 min after birth were significantly lower for the infants born from the mothers whose vitamin B-6 intake was low and EALAT stimulation was higher than the infants born from the mothers whose vitamin B-6 intake and EALAT AC values were both normal.

Animals studies have shown that vitamin B-6 restriction during gestation, either marginal or severe, affects synaptic density in the caudate/putamen, the size of the dendritic field of Purkinje cells in the cerebellum, and synaptogenesis and neuron differentiation in the neocortex[13][14][41][42].

Conclusion

1) The average vitamin B-6 intake of Korean pregnant women was 1.79mg/day, which was lower than the recommended level for pregnant women(2.2mg/day). Thirty eight percent of the pregnant subjects consumed vitamin B-6 less than the RDA level.

2) When expressed per gram of protein, pregnant participants consumed 0.017mg vitamin B-6 per gram of protein, which was slightly lower than the average consumption of the US pregnant women(0.018~0.020mg vitamin B-6/g protein).

3) Plant food was found to be a major contributor(71.8%) to the total intake of vitamin B-6 of the pregnant subjects. Vegetables & fruits, cereals & grains, and legumes provided 40.7%, 28.1%, and 2.8%, respectively of the total vitamin B-6 intake.

4) Twenty-two percent of the subjects voluntarily consumed prenatal nutritional supplements containing vitamin B-6. The most frequently used prenatal supplement was Materna of the Lederle Company which contains 4.86mg of pyridoxine · HCl(3.0mg pyridoxine) per tablet.

5) Results from the 172 cord blood EALATAC analyses showed that 32% of EALATAC values were greater than 1.25, which suggests that the prevalence of vitamin B-6 inadequacy might be significant among Korean newborn infants.

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= 국문초록 =

모체의 비타민 B-6 섭취와 신생아의 비타민 B-6 영양상태

호서대학교 식품영양학과, 이화여자대학교 의과대학 소아과**

장남수 · 김경희** · 정혜경 · 송은승

여러 인구집단 가운데 임신부, 수유부, 영유아의 비타민 B-6 영양상태가 문제시되고 있다. 본 연구에서는 임신부 98명의 비타민 B-6 섭취량을 조사하고 이들에게서 출생된 아기와 다른 아기들(총 아기수 : 172 명) 의 재태혈 적혈구 알라닌 아미노기전이효소 활성계수(Erythrocyte alaninc aminotransferase activity coefficient)을 측정하여 모체의 비타민 B-6 섭취량과 출생시 신생아의 비타민 B-6 영양상태를 비교 검토하고자 수행되었다. 임신부의 비타민 B-6 섭취량은 1.79mg± 0.88 로 미국임신부를 위한 권장량의 81.4% 에 달하는 수준이었다. 조사대상자의 38% 에 달하는 임신부가 임신부의 비타민 B-6 권장량(2.2mg/day) 보다 적게 섭취하였다. 비타민 B-6 섭취량을 단백질 섭취량으로 나눈 비율은 0.017mg/g 단백질이었다. 임신부들이 섭취한 총 비타민 B-6의 71.8% 가 생체이용율이 낮다고 알려져 있는 채소및 과일류(40.7%), 곡류(28.1%), 두류(2.8%) 등 식물성 식품이었다. 조사대상 신생아의 32% 에 해당되는 재태혈 적혈구 EALATAC 값이 1.25 이상으로 비타민 B-6 상태가 낮게 나타났다. 모체의 비타민 B-6 섭취량을 4분위로 나누어 신생아의 재태혈 EALATAC 와 비교했을때 섭취량이 높은 상위 1/4그룹에 속하는 EALATAC 값이 하위 1/4 그룹에 비해 유의적으로 낮은 것으로 보아 모체의 비타민 B-6 섭취량이 신생아의 B-6 영양상태에 영향을 미친다고 볼 수 있다.