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Vitamin B-6 Status of Mothers : Relation to Condition of the Newborn and the Neonate

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ABSTRACT

Vitamin B-6 status parameters of mothers were assessed in relation to the condition of the infant at birth and during the neonatal period.

Parameters were assessed at birth and then weekly in 18 mother-infant pairs during the neonatal period ; mothers were supplemented postnatally with 2 or 27 mg PN-HCl/d. Vitamin B-6 inadequacy in the 2 mg supplemented group was suggested by the vitamin status parameters. Mothers whose infants had unsatisfactory Apgar scores at 5 min after birth(<7) had lower vitamin B-6 status parameters than mothers whose infants were scored satisfactory. Also, infants who scored unsatisfactory at birth and whose mothers were supplemented with the low level of PN had significantly lower vitamin B-6 status parameters at 7 days of age than infants who scored satisfactory. Infants scored unsatisfactory showed some beneficial effects in both vitamin B-6 status and growth associated with the higher level of maternal postnatal vitamin B-6 supplement. In summary, the mother's prenatal and postnatal vitamin B-6 intake were significantly related to the condition of her infant at birth and during the neonatal period, respectively.

KEY WORDS : apgar scores · neonate · newborn · pyridoxal-5'-phosphate · supplement · vitamin B-6 status.

Introduction

Vitamin B-6 requirements increase during pregnancy due to estrogen induction of vitamin B-6 requiring enzymes, increased amino acid turnover, fetal demand for the vitamin, and increased maternal metabolic requirements[1-4]. Recent evidence indicates that vitamin B-6 inadequacy may nagatively affect the outcome of human pregnancy. Three published reports[5-7] provide evidence that vitamin B-6 nutriture of the mother during pregnancy may influence significantly the Apgar scores of her infant taken at one minute after birth. Differences related to PN supplementation have not been observed in Apgar scores taken 5 min after birth, a score that may be more indicative of long-term health problems of infants[7]. Beneficial effects of maternal pyridoxine supplementation on the condition of the newborn have been shown by improved Apgar scores at 1 min after birth and decreased blood oxygen affinity[8]. Tomesvari et al.[8] concluded that PN supplementation may influence favorably the oxygen trans-

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port function in blood of newborns, and that this may be especially advantageous in the early postnatal adaptation of newborns.

The American Academy of Pediatrics(AAP) Committee on Nutrition recommended that human milk be used as the reference standard for infant feeding[9]. Yet, vitamin B-6 levels in breast milk and intakes of breastfed infants are known to parallel maternal intakes of the vitamin[10][11]. Even though maternal vitamin B-6 intakes approximated the recommended dietary allowance for lactation, their breastfed infants did not meet the RDA for vitamin B-6 of 0.3mg/d[12][13].

Few studies have included vitamin B-6 intake information, biochemical indices of vitamin B-6 nutriture, and information regarding the condition of the infant at birth. This study evaluated the vitamin B-6 status of lactating mothers during the first month postpartum and examined the relation of their vitamin B-6 status to the condition of their infants at birth and during the neonatal period.

Materials and Methods

Suject Selection

Eighteen lactating women, 18yr of age or older, and their term infants(38－42 wk gestation) were recruited to participate in this study. Mother-infant pairs were excluded whenever newborns presented with anomalies, e.g. cardiac defects, inborn errors of metabolism or small for gestational age. All infants were breastfed. Thus study infants were normal and full term, 6 of whom had minor respiratory dysfunction in the first 24 hours after birth. All procedures were approved by both the hospital and the University Committees on the Use of Human Subjects.

Experimental Design

The experimental period of this study was the first 28 days of lactation. Eighteen lactating mothers who delivered term infants were assigned alternately to one of two vitamin B-6 treatment groups. Twelve mothers in one treatment group received daily a commercial prenatal supplement containing 2.0mg PN-HCl(1.7mg PN) ; the other group of six mothers received daily the commercial supplement containing 2.0mg PN-HCl and a second tablet containing 25mg PN-HCl(20.6mg PN). This level of supplementation was selected on the basis of being more than 10-fold the RDA (1989) for lactating women and available over-the-counter. Apgar score is widely used in evaluation of the newborn since it has been found to correlate with the clinical prognosis of the infant during the first few hour of life. Apgar scoring system uses a ten-point scale to rated newborns with regard to five criteria(heart rate, respiratory effort, muscle tone, reflex irritability color)[7]. At one and five minutes after birth, a specially trained neonatal nurse recorded Apgar ratings of infants on a precoded form. Samples of venous blood were collected from infants and mothers on 0, 7, 14 and 28 days. Samples of milk were collected from each mother on the same day as blood collection.

Dietary Analysis

A 24-hr dietary recall was obtained weekly for each subject by a trained interviewer and with the use of food models. Nutrient intakes, estimated from foods included in the diet records, were calculated by use of a food composition computer program[14].

Estimation of Milk Volume and Vitamin B-6 Intakes of Infants

Two methods have been used to measure the volume of milk intake of breastfed infants : total expression and thest weighing[15]. Neither method provides exact information but each provides approximate values. Test weighing was used in this study since it is less invasive to the lactation process. Mothers were provided in their homes with an electronic pediatric scale(K-Tron, Inc., Model DS-1, Scottsdale, AZ 85260) accurate to one gram and were instructed in the techniques to be used in test weighing and recording data. Test weighing was done on the same days that milk samples were collected. The infant was weighed before and after each feeding without a change in clothes. The increase in weight of the infant after feeding represents the weight of milk consumed. Weight of the milk was converted to volume by use of specific gravity of 1.031.

Vitamin B-6 intake from breast milk was calculated by multiplying the volume of breast milk intake at each feeding by the vitamin B-6 concentration of milk samples collected at each feeding. Values were summed for 24 hr periods to obtain the daily vitamin B-6 intakes of infants.

Collection of Milk and Blood Samples

Milk : Mothers collected 5−10mL of fore milk at each infant feeding during one 24-hr period weekly. Previous work showed no significant differences in the vitamer concentrations in fore and hind milk samples[16]. Milk samples were expressed manually or pump(Loyd-B-Pump) after milk "let-down" into brown plastic snap cap vials under subdued light and were frozen immediately in the home. Within 2 days, samples were transferred frozen to the laboratory where they were stored at −30℃ until analyzed.

Blood : After a 10 hr overnight fast and prior to vitamin B-6 supplementation, a 10mL sample of blood was obtained by a medical technician from the antecubital vein of the nondominant arm of the mother. Samples were collected at delivery and at 7, 14 and 28 days postpartum. At the time of delivery a 10mL sample of cord blood was obtained in lieu of infant blood. A 2mL blood sample was collected from the infants at ages 7, 14 and 28 days by venipuncture by a medical technician at the hospital. Blood samples were centrifuged ; plasma was removed in subdued light and frozen at −30℃ until analysis for B-6 vitamers. After separation from plasma and the buffy coat, erythrocytes were washed three times with 0.85% saline solution, centrifuged at 3200 rpm for 30 min and then resuspended. Aliquots of the suspended cells were added to the final incubation mixtures for anlysis of alanine aminotransferase activity.

Biochemical Analysis

The vitamer forms of vitamin B-6 in plasma, milk and erythrocytes were measured by the use of ion-pair reverse-phase high-performance liquid chromatography[17-19]. Aliquots of resuspended erythrocytes(0.5ml) were mixed with 1.0 mL of 10% (wt/v) metaphosphoric acid and centrifuged at 0−5℃ to precipitate portein. Protein-free supernatants from erythrocytes were extracted three times with one volume of ether to remove lipids and filtered(0.45μm pore size polysulfone filters, Gelman Sciences). Plasma was deproteinized by addition of 10% trichloroacetic acid, centrifugation and filtration. Chromatographic analyses were performed with a Rainin HP/HPX Drive Module(Rainin Instrument Co., Woburn, MA), sample injection valve(Rheodyne, Model 7125), ultrasphere IP C-18 column(Beckman Instruments, San Ramon, CA), a fluorescence detector (Model LS40, Perkin Elmer, Norwalk, CT), a Dynamax HPLC Method Manager Integration Software program and a MacIntosh SE computer. E-ALAT activities of erythrocytes were assayed by the method of Woodring and Storvick[20]. Activities were also determined after the addition of 100μg PLP to the assay medium in order to calculate the percent in vitro stimulation of E-ALAT by PLP[21].

Total protein content of breast milk samples was determined by the method of Lowry et al using human serum albumin as a standard[22]. Plasma alkaline phosphatase activity was measured kinetically on 20μL plasma by the catalyzed hydrolysis of p-nitrophenylphosphate to p-nitrophenol and inorganic phosphate[23][24]. One unit of activity was defined as the amount of enzyme which produced 1 μmol p-nitrophenol per minute under the conditions of the assay.

Recovery and Precision Tests

Recoveries of B-6 vitamers were determined by spiking plasma samples with vitamers[(ng) PLP, 25 ; PMP, 17.5 ; PL, 25 ; PN, 17.5 ; and PM, 17.5] prior to the deproteinization step and comparing the increase in vitamer concentration in relation to that added. Recoveries of PLP, PMP, PL, PN and PM added to plasma by HPLC method averaged 93± 4, 104± 6, 99± 7, 106± 10 and 109± 12(mean± SD)%, respectively.

Within assay reproducibility was determined by analysis of three replicates of plasma samples. The precision of PLP, PMP, PL, PN and PM expressed as coefficient of variation is 3.2± 0.1, 4.0± 0.2, 3.7± 0.2, 3.4± 0.1 and 2.9± 0.1(mean± SD)%, respectively. Coefficient of variability (CV) was calculated by dividing the SD by mean×100.

Assessment of Infant Growth

Weight and length measurements of infants were obtained weekly during the 28-day study. All measurements were made by one individual, a trained pediatric nurse. A K-Tron electronic balance(Model DS-1, Scottsdale, AZ 85260) was used to obtain infant weights. Lengths were measured by use of a portable tape board equipped with a head and foot board. These measurments were compared with growth reference percentiles and Z-scores(standard deviation from the reference mean) published by the National Center for Health Statistics(NCHS)[25].

Statistical Analysis

Statistical analysis of data was done in conjunction with the Department of Statistics, Purdue University ; programs from the Statistical Analysis System(SAS) were used[26]. Infant weight Z-scores were calculated by use of software programs based on the growth reference curves developed by the National Center for Health Statistics and Center for Disease Control(CDC)[27].

Two-way analysis of variance was done to determine the effects of different level of vitamin B-6 supplementation on plasma PLP levels, percent stimulation of E-ALAT by PLP added in vitro and plasma B-6 vitamers concentrations of mothers at different stages of lactation. Any group mean giving a significant F value($p<0.05$) was further tested to distinguish which means were statistically different by use of the Newman-Keuls sequential test for groups of equal/unequal size. Pearson correlation coefficients were also calculated from regression analysis of certain veriables. Student's t-test was done to determine the effect of different levels of maternal PN-HCl supplementation on B-6 vitamer levels and alkaline phosphatase activity in plasma.

Multiple regression was used as a model building tool and in the process, many different models were examined for the prediction of weight changes of the neonate. Essentially all subsets of variables were examined as candidate predictors and lists of subsets, ordered by R^2, were generated within the subset size by use of the statistical software package, SAS Procedure RSQUARE. The CP criterion[28] was used to select subset models. The methods allowed attention to be focused on those models which had the greatest potential for answering the research questions.

Results

Maternal Nutrient Intake

Nutrient intakes of mothers were estimated by the use of four 24-hr dietary recalls collected weekly during the first month of lactation. The records indicated that the recommended dietary allowances(1989) for energy, protein, vitamin C, thiamin, riboflavin and calcium were met whereas vitamin A, iron, zinc, magnesium and vitamin B-6 intakes were less than recommended. Mean calculated prenatal/postnatal intake of vitamin B-6 was 1.8mg/day or approximately 82% of the RDA for pregnancy(2.2mg) and 86% of the RDA for lactation(2.1mg). When expressed as ratios of the vitamin to protein and energy intake, the mean vitamin B-6 intake was 23.5± 8.2μg/g protein and 0.82± 0.20mg/1000kcal. Total vitamin B-6 intake of the subjects was increased markedly postnatally by daily supplements of 27mg PN-HCl(22.2mg PN).

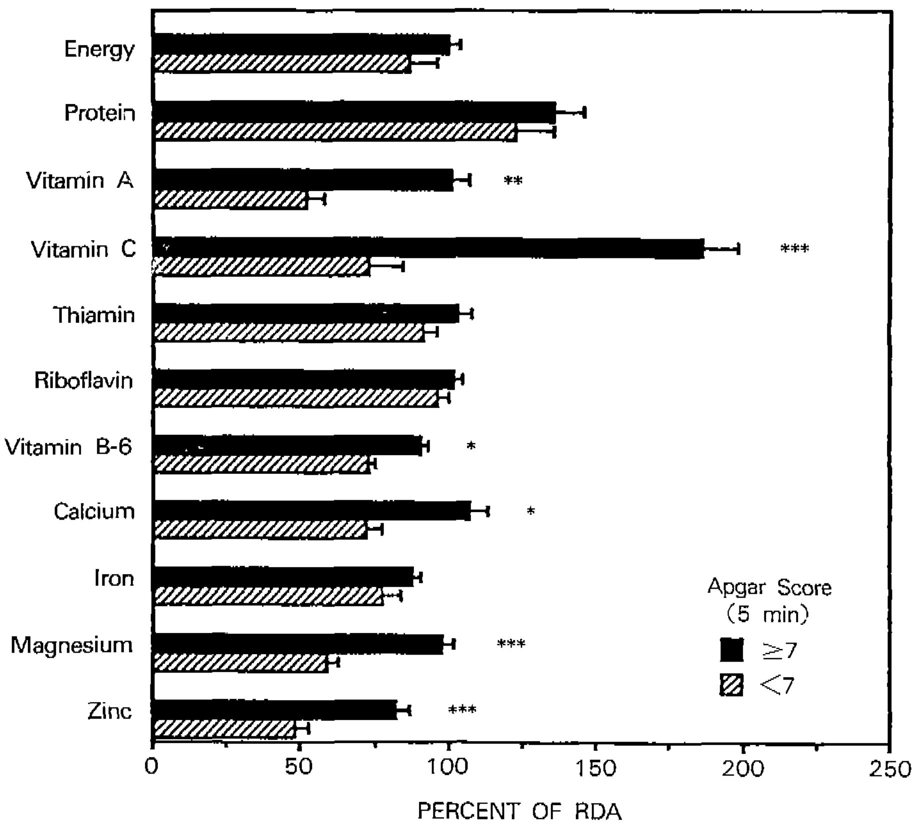

Fig. 1. Mean daily nutrient intakes of mothers from dietary sources expressed as percentages of their Recommended Dietary Intakes(12). *$p<0.05$, **<0.01, ***$p<0.001$ significantly different from unsatisfactory group.

Maternal Vitamin B-6 Status at Delivery and Infant Condition at Birth

Mothers whose infants received unsatisfactory Apgar scores(<7) at 5 min after birth(n=4) had significantly lower dietary intakes of vitamin A, vitamin C, vitamin B-6, calcium, magnesium and zinc than those whose infants had satisfactory scores(≥7)(n=14)(Fig. 1). The values for vitamin B-6 intake are attributed primarily to the use of prenatal supplements. Data for prenatal supplement use were obtained retrospectively from mothers. The regularity of supplement use was not monitored. The significantly lower vitamin B-6 intakes(diet+prenatal supplements) of mothers whose infants received unsatisfactory Apgar scores was consistent with the lower levels of the vitamin observed in maternal plasma.

PLP levels in maternal plasma at delivery and cord plasma were significantly lower among mothers whose infants had unsatisfactory Apgar scores(<7) at 5 min than levels in plasma of those whose infants were rated satisfactory(Table 1). Plasma PLP values were 42.5 and 24.5 nmol/L for mothers of infants with satisfactory and unsatisfactory Apgar scores, respectively. The ratio of PLP in cord to maternal plasma was higher for mothers whose infants had unsatisfactory Apgar scores than for those who had satisfactory scores. Also, mothers whose infants received unsatisfactory Apgar scores had significantly higher E-ALAT stimulation, suggesting vitamin B-6 inadequacy, than those whose infants had satisfactory scores.

The difference between the mean birth weights of the two groups of infants(satisfactory and unsatisfactory Apgar scores) was not statistically significant($p>0.05$). However, mean birth length was significantly less($p<0.05$) for infants with unsatisfactory Apgar scores.

Relationships Between Prenatal Maternal Vitamin B-6 Intake and Parameters of Vitamin B-6 Status

Table 1. Relation of certain parameters of maternal vitamin B-6 status and infant anthropometry at delivery to Apgar scores of the infants at 5 minutes after birth

Maternal and infant parameters	Apgar scores		Significance
	≥7	<7	
	n=14	n=4	p
Maternal			
Prenatal vitamin B-6 intake,* mg/d	8.5 ± 0.8†	5.2 ± 1.1	<0.05
Plasma PLP, nmol/L	42.5 ± 2.8	24.5 ± 5.4	<0.02
Plasma PL/PLP ratio	0.23± 0.03	0.18± 0.02	NS
Stimulation of E-ALAT by PLP, %	11 ± 4	19 ± 2	<0.02
Infant			
Cord plasma PLP, nmol/L	178 ± 14	103 ± 8	<0.005
Cord/maternal plasma PLP ratio	4.0 ± 0.4	5.4 ± 0.9	NS
Cord plasma PL/PLP ratio	0.21± 0.03	0.15± 0.01	NS
Infant birth weight, g	3463 ± 97	3108 ± 140	NS
Z-score	0.35± 0.19	0.18± 0.12	NS
Infant birth length, cm	51.4 ± 0.4	47.1 ± 1.1	<0.05
Z-score	0.01± 0.08	−0.40± 0.15	<0.04

*Includes diet and prenatal supplement.
†Mean± SEM

Prenatal vitamin B-6 intake of the mothers was significantly correlated with PLP levels in maternal plasma(r=0.65) and cord plasma(r=0.44) (Table 2). Also, the levels of PLP in cord plasma were significantly correlated with PLP and PL levels and with PL/PLP ratios in maternal plasma (Table 3).

Interrelations among the maternal blood measurements were assessed. Maternal plasma PL/PLP ratio was significantly positively correlated with plasma PLP and PL levels and erythrocyte PLP concentrations whereas maternal erythrocyte PLP levels were positively correlated with plasma PL levels and were negatively correlated with stimulation of E-ALAT by PLP.

Vitamin B-6 Concentration in Milk

Throughout the neonatal period, total vitamin B-6 levels in milk paralleled the level of postnatal maternal vitamin B-6 supplementation ; levels were lower in milk of mothers who received 2mg PN-HCl/d than those who received 27mg PN-HCl/d(Fig. 2).

Vitamin B-6 Intake of Infants

Levels of postnatal maternal vitamin B-6 supplementation did not affect the volume of milk intake of infants. Vitamin B-6 intake of all groups of infants increased during the neonatal period

Table 2. Correlation of prenatal vitamin B-6 dietary intakes of mothers to certain parameters of their vitamin B-6 status at delivery

Maternal measurements	Prenatal maternal dietary intake of vitamin B-6, mg/d	
	Pearson correlation coefficient	Significance
n=18	r	p
Cord plasma PLP, nmol/L	0.44	<0.05
Cord plasma PL/PLP	0.43	<0.05
Plasma PLP, nmol/L	0.65	<0.02
Plasma PL/PLP	0.50	<0.04

Table 3. Correlation of cord plasma PLP concentration to vitamin B-6 status measurements of mothers at delivery and interrelations among the maternal measurements

Maternal Measurements	Cord plasma PLP, nmol/L	
	Pearson correlation coefficient	Significance
n=18	r	p
Plasma PLP, nmol/L	0.58	<0.01
Plasma PL, nmol/L	0.82	<0.0001
Plasma PL/PLP ratio	0.59	<0.009
Erythrocyte PLP, nmol/L	0.59	<0.009
Stimulation of E-ALAT by PLP, %	−0.53	<0.02
	Maternal plasma PL/PLP ratio	
Plasma PLP, nmol/L	0.59	<0.009
Plasma PL, nmol/L	0.90	<0.0001
Erythrocyte PLP, nmol/L	0.61	<0.007
	Maternal erythrocyte PLP, nmol/L	
Plasma PL, nmol/L	0.63	<0.005
Stimulation of E-ALAT by PLP, %	−0.48	<0.04

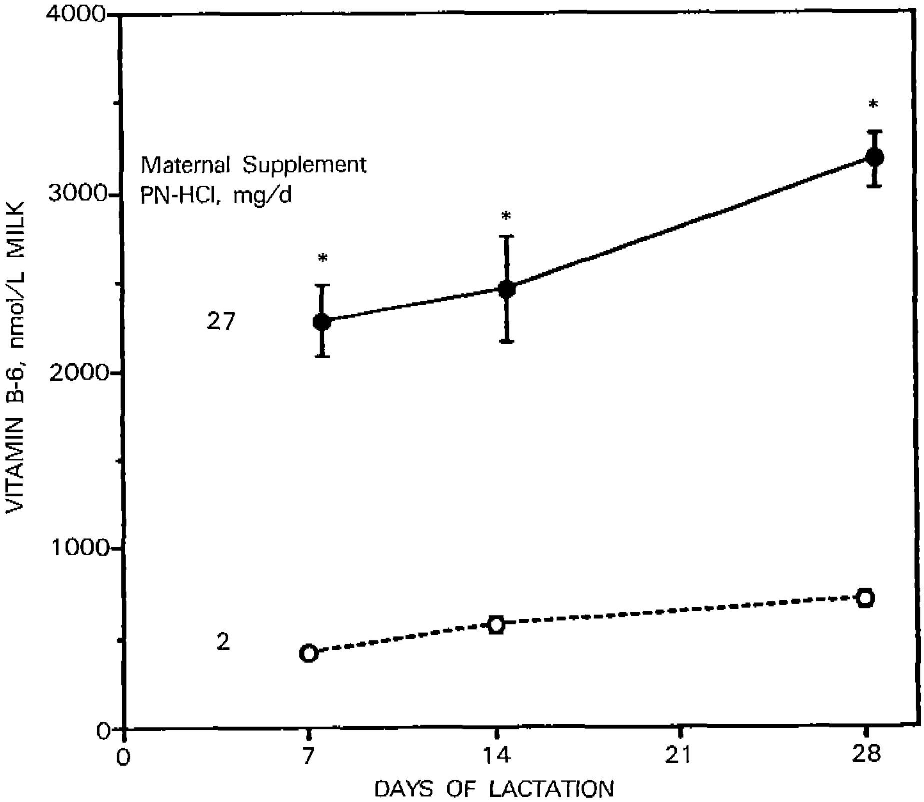

Fig. 2. Vitamin B-6 concentration in milk from mothers supplemented with two different levels of vitamin B-6 during the neonatal period. Vertical bars represent mean± SEM. *p<0.0001 significantly different from 2 mg supplemented counterpart.

(Fig. 3). Vitamin B-6 intakes of breastfed infants paralleled their mother's level of supplementation and were markedly higher when their mothers were supplemented with 27mg PN-HCl/d.

Most breastfed infants of mothers supplemented postnatally with 2mg PN-HCl/d did not meet the current RDA. Even when maternal intakes of vitamin B-6 exceeded ten times the RDA for lactation, intakes of infants who had unsatisfactory Apgar scores at 5min(<7) did not meet the RDA during the 28 days neonatal period.

Relationships Between Infant Intake and Parameters of Vitamin B-6 Status

Parameters of vitamin B-6 status of infants at 7 days of age classified according to their Apgar scores at 5 min after birth are shown in Table 4. Infants who had unsatisfactory Apgar scores at 5 min(<7) and whose mothers were supplemented with 2mg PN-HCl/d(Group B) had significantly lower intakes of vitamin B-6 and protein, lower plasma and erythrocyte PLP levels and higher percent stimulation E-ALAT compared to those who had satisfactory scores(Group A). These findings suggested inadequacy of vitamin B-6 in these infants at 7 days of age.

Infants who had unsatisfactory Apgar scores at 5 min(<7) and whose mothers were supplemented with 27mg PN-HCl/d(Group C) had higher intakes of vitamin B-6 and vitamin B-6/protein

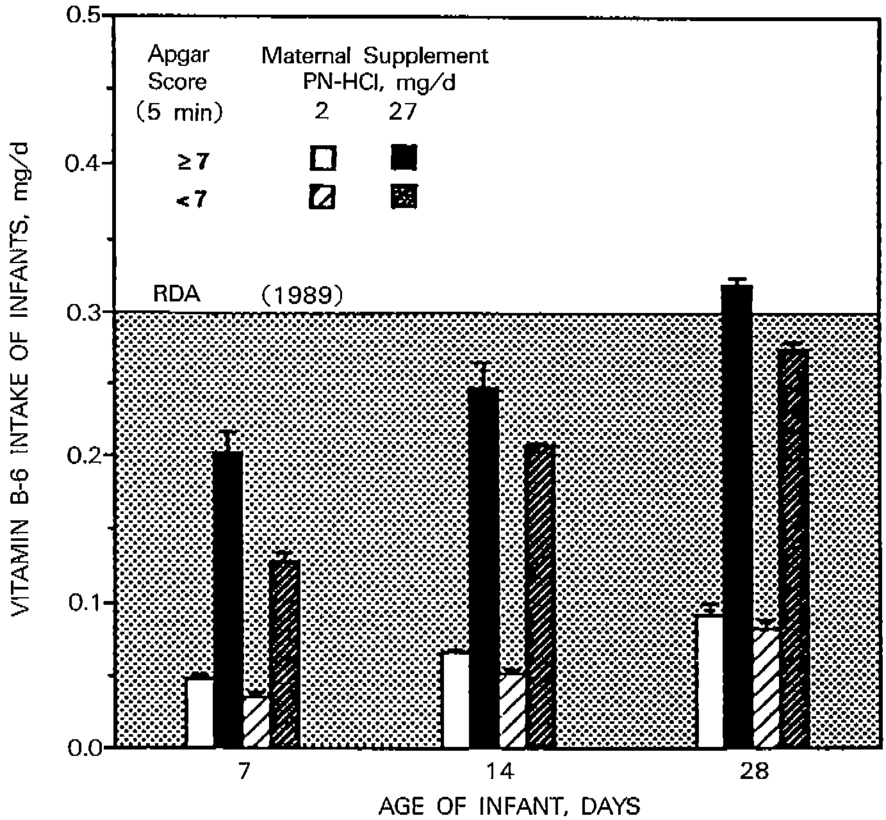

Fig. 3. Vitamin B-6 intakes of breastfed infants of mothers supplemented with different levels of vitamin B-6 during the neonatal period. Vertical bars represent mean± SEM. Shaded area represents intakes below the current RDA(1989) of vitamin B-6 intake/day for infants, 0－6 mo of age.

ratio, higher plasma PLP and PL and erythrocyte PLP and similar values of plasma PL/PLP ratio, alkaline phosphatase activity and percent stimulation E-ALAT compared to those who had satisfactory scores and whose mothers were supplemented with 2mg PN-HCl/d(Group A)(Table 4). In these preliminary findings the number of subjects was small ; however, marked differences between the two levels of maternal PN supplements on the vitamin B-6 status of infants who had unsatisfactory Apgar scores at 5min after birth suggested that the high level of supplement was beneficial to these infants.

The levels of plasma PLP, PL and PL/PLP ratio and erythrocyte PLP were significantly positively correlated, whereas plasma alkaline phosphatase and percent stimulation of E-ALAT were negatively correlated with the vitamin B-6 intake of infants(Table 5).

Effect of Postnatal Vitamin B-6 Supplementation on Maternal Vitamin B-6 Status

The mean values of the biochemical indicators of maternal vitamin B-6 status for the two levels of supplement groups are shown in Table 6. During the first month of lactation, plasma and erythrocyte PLP concentrations of mothers who received supplements of 2mg PN-HCl/d were significantly less than those who received 27mg PN-

Table 4. Vitamin B-6 status measurements of infants at 7 days of age in relation to their Apgar scores at 5 minutes after birth and maternal postnatal vitamin B-6 intake

Infant measurements at 7 days of age	Group A	Group B	Group C	Significance	
	Postnatal maternal vitamin B-6 intake, mg/d				
	3.3*	3.3*	24.3†		
	Infant Apgar scores, 5 min after birth				
	≥7	<7	<7	AVB	AVC
Vitamin B-6 intake, μg/d	48 ± 3‡	35 ± 4	129 ± 12	<0.05	<0.001
Protein intake, g/kg/d	3.1 ± 0.2	2.4 ± 0.1	2.6 ± 0.3	<0.05	NS
Vitamin B-6/protein intake, μg/g	4.1 ± 0.2	3.7 ± 0.8	17.7 ± 1.2	NS	<0.001
Plasma PLP, nmol/L	44 ± 4	31 ± 3	86 ± 9	<0.05	<0.01
Plasma PL, nmol/L	8 ± 2	5 ± 1	17 ± 4	NS	<0.01
Plasma PL/PLP ratio	0.24± 0.05	0.18± 0.01	0.25± 0.03	NS	NS
Plasma alkaline phosphatase, U/L	118 ± 6	147 ± 19	124 ± 14	<0.05	NS
Erythrocyte PLP, nmol/L	116 ± 4	89 ± 2	215 ± 12	<0.004	<0.05
Stimulation of E-ALAT by PLP, %	10 ± 2	18 ± 1	9 ± 3	<0.05	NS

*Diet plus 1.7 mg PN equivalents in 2mg PN-HCl supplement.
†Diet plus 22.2 mg PN equivalents in 27 mg PN-HCl supplement.
‡Mean± SEM.

Table 5. Correlation of vitamin B-6 intakes of infants to certain vitamin B-6 status measurements at 7 days of age

Infant measurements	Vitamin B-6 intakes of infants, μg/d	
	n=18	
	r*	p
Plasma PLP, nmol/L	0.82	<0.0001
Plasma PL, nmol/L	0.72	<0.0001
Plasma PL/PLP ratio	0.68	<0.001
Plasma alkaline phosphatase activity, U/L	−0.47	<0.04
Erythrocyte PLP, nmol/L	0.88	<0.0001
Stimulation of E-ALAT by PLP, %	−0.52	<0.001

*Pearson correlation coefficient.

HCl/d. High percent stimulation of E-ALAT was observed at 7, 14 and 28 days of lactation in mothers supplemented with 2mg PN-HCl/d.

PLP and PL were the major vitamin B-6 compounds in plasma. The mean plasma PLP and PL levels of mothers supplemented with 27mg vitamin B-6 were increased by 81 and 89% respectively, compared to the 2mg supplement group (Table 6). The plasma PL/PLP ratios of mothers who received supplements of 2mg PN-HCl/d were significantly lower than those of the higher supplement group. Alkaline phosphatase activity in the two groups was not significantly($p>0.05$) different.

Maternal plasma PLP and PL levels and PL/PLP ratio were positively correlated whereas percent stimulation of E-ALAT was negatively correlated with vitamin B-6 supplementation during first month of lactation. Plasma alkaline phosphatase activity was not significantly related to vitamin B-6 supplementation(Table 7).

Maternal vitamin B-6 nutritional status, assessed by plasma PLP and PL levels, plasma PL/PLP ratio and percent stimulation of E-ALAT were significantly related to the vitamin B-6 concentration of milk(Table 8).

Growth of Infants

Growth performance of infants was evaluated by percentile and Z-scores of weight-for-age and

Table 6. Relation of vitamin B-6 intake to plasma and erythrocyte measurements of mothers at different stages of lactation

Stage of lactation	Maternal vitamin B-6 intake, mg/d		Significance
	3.3*	24.3†	
days	n=12	n=6	p
	Plasma PLP, nmol/L		
0	33 ± 7‡	37 ± 10	NS
7	40 ± 7	142 ± 21	<0.0008
14	37 ± 9	241 ± 24	<0.0001
28	33 ± 4	248 ± 24	<0.0001
	Plasma PL, nmol/L		
0	8 ± 2	10 ± 4	NS
7	8 ± 2	57 ± 14	<0.004
14	10 ± 2	97 ± 20	<0.001
28	10 ± 4	93 ± 13	<0.0002
	PL/PLP		
0	0.20± 0.06	0.24± 0.07	NS
7	0.19± 0.04	0.40± 0.08	<0.0005
14	0.24± 0.04	0.41± 0.09	<0.001
28	0.23± 0.04	0.38± 0.04	NS
	Plasma alkaline phosphatase, U/L		
0	80 ± 5	69 ± 12	<0.08
7	63 ± 10	69 ± 14	NS
14	68 ± 5	74 ± 20	NS
28	59 ± 14	52 ± 14	NS
	Erythrocyte PLP, nmol/L		
0	99 ± 10	106 ± 18	NS
7	87 ± 6	373 ± 38	<0.0001
14	94 ± 25	488 ± 50	<0.0001
28	82 ± 9	495 ± 43	<0.0001
	Stimulation of E-ALAT by PLP,% §		
0	18 ± 1	10 ± 3	<0.03
7	21 ± 3	4 ± 2	<0.01
14	18 ± 3	7 ± 1	<0.004
28	14 ± 6	4 ± 2	<0.001

*Diet plus PN equivalents in 2 mg PN-HCl supplement.
†Diet plus PN equivalents in 27 mg PN-HCl supplement.
‡Mean± SEM.
§Normal values<16.0%

length-for-age in relation to the National Center for Health Statistics reference(NCHS)[25].

During the neonatal period, the pattern of change in weight and length Z-scores of infants who had unsatisfactory Apgar scores at 5 min after birth and those who had satisfactory scores were similar. Weekly increases in weight and length Z-scores were generally greater for infants who had satisfactory Apgar scores and whose mothers were supplemented with 27mg PN-HCl/d compa-

Table 7. Correlation of postnatal maternal vitamin B-6 intake to certain vitamin B-6 status measurements at different stages of lactation

Maternal measurements	Postnatal maternal vitamin B-6 intake,* mg/d		
	Stage of lactation	Pearson correlation coefficient	Significance
N=18	days	r	p
Plasma PLP, nmol/L	7	0.94	<0.0002
	14	0.95	<0.001
	28	0.96	<0.0006
Plasma PL, nmol/L	7	0.82	<0.006
	14	0.85	<0.01
	28	0.93	<0.003
Plasma PL/PLP	7	0.64	<0.06
	14	0.44	<0.30
	28	0.72	<0.06
Stimulation of E-ALAT by PLP, %	7	−0.86	<0.003
	14	−0.77	<0.04
	28	−0.58	<0.09
Plasma alkaline phosphatase activity, U/L	7	0.12	<0.70
	14	0.30	<0.52
	28	0.39	<0.31
Milk total vitamin B-6, nmol/L	7	0.98	<0.0001
	14	0.96	<0.0001
	28	0.99	<0.0001

*Diet plus PN equivalents in PN-HCl supplements.

Table 8. Correlation of vitamin B-6 levels in milk to certain vitamin B-6 status measurements of mothers

Maternal measurements*	Total vitamin B-6 in milk,* nmol/L	
	Pearson correlation coefficient	Significance
n=18	r	p
Plasma PLP, nmolL	0.97	<0.0001
Plasma PL, nmol/L	0.93	<0.0001
Plasma PL/PLP	0.73	<0.001
Erythrocyte PLP, nmol/L	0.98	<0.0001
Stimulation of E-ALAT by PLP	−0.86	<0.0001
Vitamin B-6 intake, mg/d	0.98	<0.0001

*Means of measurements made on 7, 14 and 28 days of lactation.

Table 9. Simple and multiple regression of maternal and infant variables for predicting changes of weight expressed as Z-score

Explanatory variable	Regression coefficient± SE	p	R^2
n=18	Response variable : Weight change from birth to 7 days, Z-score		
	Multiple regression		
Maternal vitamin B-6 intake, mg/d	0.02 ±0.01	0.02	0.69
Apgar score at 5 min after birth	0.09 ±0.04	0.07	
Birth weight, Z-score	−0.002±0.001	0.11	
Intercept	−0.26 ±0.42		
	Response variable : Weight change from 7 to 28 days, Z-score		
n=18	Multiple regression		
Apgar score at 5 min after birth	0.07 ±0.01	0.002	0.79
Maternal vitamin B-6 intake, mg/d	0.003±0.001	0.12	
Birth weight, Z-score	−0.04 ±0.03	0.15	
Intercept	−0.49 ±0.11		
Apgar score at 5 min after birth	0.06 ±0.01	0.0002	0.89
Infant plasma PLP, nmol/L	0.002±0.001	0.006	
Intercept	−0.45±0.07		

red to other groups.

Certain maternal and infant characteristics were examined as predictors of infant weight changes expressed as Z-scores(Table 9). By simple regression analysis, maternal vitamin B-6 intake showed a significant relationship to weight change from birth to 7 days of age. Multiple regression analysis was then used to obtain the most significant model for explaining the variation in changes of weight from birth to 7 days and birth to 28 days of age. The combination of three variables : maternal vitamin B-6 intake, Apgar score at 5 min after birth and birth weight, increased the power of the prediction of weight change from birth to 7 days, accounting for 69% of the variation ; most of which was related to maternal vitamin B-6 intake. The same three-variable model accounted for 79% of the variation in weight change observed from 7 to 28 days of age ; most of the variation was associated with Apgar scores at 5 min after birth.

Weights at birth and at 28 days of age, expressed as percent of the 50th percentile(NCHS), were positively related(r=0.78). Birth weight percentile was negatively related(r=−0.62) to the change in weight percentile from 7 to 28 days of age. Most infants increased in weight percentiles during the first 28 days ; this change was greater for infants who were in the lower percfentiles at birth.

Discussion

Maternal Vitamin B-6 Nutriture and Condition of the Newborn

The relationship of maternal vitamin B-6 status to the condition of the newborn is not clear-cut because the fetus meets its need for vitamin B-6 by extracting the vitamin from the mother via the placenta. The Apgar score is widely used in the evaluation of the newborn since it has been found to correlate with the clinical prognosis of the infant during the first few hours of life[29]. Infant mortality has been related inversely to Apgar scores at 1min ; the prognosis for survival worsens the longer the Apgar score remains low[30]. Very low scores at both 1 and 5 min after birth have been associated with chronic neurologic disability in infants[31].

Three reports[5-7] provide evidence that vitamin B-6 nutriture of the mother during pregnancy is associated significantly with the Apgar score of her infant at birth. Roepke and Kirksey[5] first reported that mothers whose infants received unsatisfactory Apgar scores at 1 min(<7) had significantly lower intakes of vitamin B-6, lower B-6 levels in serum at delivery and lower vitamin B-6 concentrations in milk at 3 and 14 days postpartum than mothers whose infants received satisfactory Apgar scores. Schuster et al[6] observed low Apgar scores at 1 min after birth for infants whose mothers showed vitamin B-6 inadequacy as indicated by high stimulation of erythrocyte ALAT compared to scores for infants whose mothers had normal stimulation values.

Subsequently, Schuster et al[7] showed that Apgar scores at 1 min after birth were significantly higher for infants whose mothers consumed 7.5mg or more of supplemental PN-HCl daily during pregnancy than for infants of mothers who consumed 5mg or less. Until the present study, however, differences related to maternal vitamin B-6 supplementation have not been observed in Apgar scores taken 5 min after birth, a score that may be more indicative of long-term health problems in the infant.

In the present study, mothers whose infants received unsatisfactory Apgar scores(<7) at 5 min after birth had significantly lower dietary intakes of vitamin A, vitamin C, vitamin B-6, calcium, magnesium and zinc than those whose infants had satisfactory scores(≥7). The dietary data suggested the possibility of multinutrient inadequacies. Even though the focus of this study was on a single nutrient, vitamin B-6, this does not preclude the possibility that other nutrients were also associated with the outcome variables measured. Significantly lower vitamin B-6 intakes(diet plus prenatal supplements) of mothers whose infants received unsatisfactory Apgar scores was consistent with the lower levels of the vitamin observed in maternal plasma.

PLP levels in maternal plasma at delivery and cord plasma were significantly lower and cord to maternal plasma PLP ratios were higher among mothers whose infants had unatisfactory Apgar scores at 5 min after birth(<7) than those whose infants were rated satisfactory. In normal pregnancy, the cord to maternal plasma B-6 vitamer ratio has been reported by Contractor and Shane[32] to be 2.5. The higher than normal ratios of cord to maternal plasma PLP observed in this study(4.0 and 5.4) concomitant with low levels of PLP in maternal plasma, suggested some drain of vitamin B-6 from the mother to the fetus. Mean estimates of prenatal vitamin B-6 dietary intake were 1.7 and 2.0 mg/d for mothers of infants with satisfactory and unsatisfactory Apgar scores, respectively. Mothers whose infants received unsatisfactory Apgar scores had significantly lower prenatal intakes of vitamin B-6 and higher erythrocyte ALAT stimulation(suggesting vitamin B-6 inadequacy) than those whose infants had satisfactory scores. These findings suggested that the condition of the newborn was related, in part, to maternal vitamin B-6 status.

Prenatal maternal PN supplementation may have a beneficial effect on the condition of the newborn. Temesvari et al[8] reported that PN supplementation influenced favorably the oxygen transport function in blood of newborns, perhaps due to its effects on 2, 3-diphosphoglycerate as well as PLP, and that this may be especially advantageous in the early postnatal adaptation of newborns. Kirksey et al[33] reported that inadequate maternal vitamin B-6 intake contributed to abnormal neonatal behavior and neurological abnormalities observed in BF infants. Neonatal behavior, quantified by the Brazelton Neonatal Behavioral Assessment Scale, indicated that consolability, appropriate build-up to a crying state and response to aversive stimuli were correlated with maternal vitamin B-6 nutriture[34]. Animal studies have demonstrated that inadequate maternal vitamin B-6 intake results in delayed and impaired neuromotor development of the offspring of rats [35][36]. The growth rate of brain is most rapid just before birth when PLP is critical to myelination and other aspects of the developing fetal nervous system[37-39].

Growth of Infants

In this study weight and length of most infants were between the 25th and 75th percentiles of the NCHS reference in spite of low intakes of vitamin B-6 relative to the RDA(1989). Weekly changes in weight and length Z-scores of infants from birth to 28 days were correlated with their vitamin B-6 intakes. Weekly increases in weight and length Z-scores of breastfed infants(Apgar scores$\geq$ 7 at 5 min) whose mothers were supplemented postnatally with 27 mg PN-HCl/d were greater than for other groups.

Proc RSQUARE procedure and multiple regression analysis were used to examine the combined effects of different maternal and infant variables in relation to infant weight changes. Postnatal maternal vitamin B-6 intake was the best single predictor of infant weight change from birth to 7 days. Apgar score at 5 min after birth was the best single predictor of infant weight change from 7 to 28 days. Greater predictive variance was accounted for when maternal vitamin B-6 intake was combined with Apgar scores at 5 min after birth and birth weight. The results of this study showed a growth advantage for breastfed infants when the maternal supplement exceeded 2 mg/d. Also in the case of infants who had unsatisfactory Apgar scores at 5 min, a maternal supplement greater than 2 mg/d was associated with a growth advantage.

Total Vitamin B-6 Levels in Milk

Concentrations of total vitamin B-6 as well as the individual B-6 vitamers in human milk have been implicated in the nutritional management of breastfed infants. Our findings agree with previous reports[10][16][40][41] that the vitamin B-6 concentration in breast milk is influenced by maternal intake of the vitamin. Lower concentrations of total vitamin B-6 and a smaller percentage of PL were observed in milk of mothers supplemented with 2 mg PN-HCl/d than those supplemented with 27 mg. PL is the predominant vitamer in milk and the most responsive to vitamin B-6 intake.

Studies on absorption rates of the individual B-6 vitamers in rats showed that PL has a two fold higher intestinal absorption rate than PN or PM[42][43]. The high percentage of PL in human milk may be beneficial to the neonate because it is absorbed rapidly and metabolized directly to PLP by PL kinase. On the other hand, PN is metabolized to PNP by PN-5'-P oxidase, a riboflavin-dependent enzyme, and the vitamer is metabolized to PLP by PL kinase.

Vitamin B-6 Intakes of Infants

Vitamin B-6 intakes of breastfed infants paralleled their mothers level of supplementation. However, even when maternal intakes of vitamin B-6 exceeded more than ten times the RDA for lactation, infant intakes did not meet the RDA of 0.3 mg/d until they reached 28 days of age. These findings agreed with previous work in this laboratory[11)44)] that a maternal supplement greater than 2 mg PN-HCl/day was needed by some mothers to insure the adequacy of vitamin B-6 intakes by their breastfed infants.

Effect of Vitamin B-6 Supplementation on Maternal Vitamin B-6 Status

In this study assessment of maternal vitamin B-6 status by both dietary and biochemical indices(plasma and erythrocyte PLP, plasma PL/PLP ratio, percentage stimulation of E-ALAT and plasma alkaline phosphatase activity) indicated considerable individual differences in the measurements.

Mean vitamin B-6 intake of all subjects was estimated to be 1.8 mg/day or 86% of the current RDA of 2.1 mg/day. Dietary intakes of vitamin B-6 were similar for mothers supplemented with different amounts of PN-HCl. Thus differences in total B-6 intakes(diet plus supplement) were attributed to differences in the amount of PN-HCl supplementation.

Plasma PLP concentrations of cord blood were significantly correlated to the amount of PN-HCl supplement consumed during the prenatal period. These findings agree with those of previous studies done in this[12)45)] and other[46)47)] laboratories. PLP concentration of maternal blood sampled at delivery has also been observed to parallel the level of prenatal PN-HCl supplement[7)32)]. In this study, this relationship was found to apply to the PLP concentration in maternal plasma at 0, 7, 14 and 28 days of lactation.

Additionally, the correlation between PN-HCl supplementation and maternal plasma PLP concentrations increased with the length of time of supplementation. This may have been related to some depletion in maternal tissue saturation of vitamin B-6 during pregnancy[7)32)] followed by gradual repletion after supplementation with the vitamin during lactation.

Maternal vitamin B-6 nutritional status, assessed by plasma PLP, plasma PL/PLP ratio and percent stimulation of E-ALAT, was a strong marker of vitamin B-6 concentration in milk. Plasma PLP and PL concentrations, and PL/PLP ratio determined at 7, 14 and 28 days of lactation were positively correlated and percent stimulation of E-ALAT by PLP was negatively correlated with mean vitamin B-6 concentrations in breast milk sampled at each feeding during a 24hr period on the same day that blood was collected. Vitamin B-6 content of breast milk has also been observed to be influenced by maternal vitamin B-6 nutritional status, assessed by serum vitamin B-6[48)] and plasma PLP concentration[45)]. Our results indicated that maternal vitamin B-6 status was improved by the use of supplemental vitamin B-6 during the first month of lactation. This was associated with a greater response to the supplement as reflected by increased concentrations of vitamin B-6 in milk.

Additionally, plasma PLP concentrations of mothers who were supplemented with 2 mg PN-HCl/d or 3.3 mg total intake(diet plus supplement calculated as PN equivalents) was lower(36± 7 nmol/L) than the levels of nonlactating women (57± 7 nmol/L)[49)50)], and appeared to provide low concentrations of vitamin B-6 reported that daily supplements between 2.5 and 4 mg PN-HCl during lactation resulted in relatively saturated concentrations of vitamin B-6 in milk.

This study supports previous findings from this laboratory[45)] that the recommended allowance of vitamin B-6 intake for lactating women is incompatible with the recommended allowance of the vitamin for infants and that the allowance for infants may be unrealistically high. Clearly, the current recommended allowance of vitamin b-6 of 2.1 mg/day for lactating mothers did not provide a sufficient level of the vitamin in breast milk to meet the current recommended allowance of 0.3 mg/d for infants who were solely breastfed.

Conclusions

In conclusion, these data showed a significant association of the mother's vitamin B-6 nutriture on the condition of her infant at birth and during the neonatal period. Preliminary findings also indicated beneficial effects of maternal vitamin B-6 supplementation during the first week postpartum on th vitamin B-6 status of infants who had unsatisfactory Apgar scores at 5 min after birth.

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= 국문초록 =

모체의 비타민 B-6 영양상태가 신생아의 건강상태와 영양상태에 미치는 영향

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모체의 임신 수유기간 동안의 vitamin B-6 영양상태가 출생시와 신생아기의 건강과 영양상태에 미치는 영향에 대해 연구하였다. 수유부를 2mg과 27mg PN-HCl 섭취하는 두 군으로 나누고 수유부ㅡ신생아의 vitamin B-6 섭취량을 생후 1개월동안(신생아기) 1주마다 측정하였고 영양상태 지표는 탄생시와 신생아기동안 1주마다 수유부와 신생아의 혈장과 적혈구의 vitamin B-6 영양상태 평가 지표를 이용해서 측정하였다.

모유의 total vitamin B-6 농도와 PL(predominant vitamer in milk)의 분포도는 모체가 vitamin B-6를 27mg PN-HCl/d 섭취한 군이 2mg PN-HCl/d 섭취한 군보다 유의적으로 높음을 보인 것은 모유속에 함유된 vitamin B-6는 모체의 vitamin B-6 섭취에 의해 반응을 보임을 알 수 있었다. 모체 영양상태가 vitamin B-6 영양 불균형일 때 임신결과에 역효과를 미치고 있음은 탄생시 5분 후에 측정하는 Apgar score가 7보다 적은 군의 모체 vitamin B-6 영양상태는 7보다 큰 군의 모체보다 양호하지 못함을 통해 알 수 있었다. 임신기간 동안의 모체의 vitamin B-6 영양상태가 신생아의 Apgar score에 유의적인 영향을 미치고 있으며 모체의 vitamin B-6 섭취에 의해 신생아의 Apgar score를 유익하게 향상시키고 vitamin B-6 영양상태(plasma PLP, PL/PLP, erythrocyte PLP, stimulation % of E-ALAT by PLP, alkaline phosphatase activity)와 성장도(weight change, Z-score)에도 유익한 영향을 미침을 고찰하였다. 유아의 vitamin B-6 권장량은 수유부의 권장량과 상응되지 않을 뿐더러 유아의 필요량이 비이상적으로 높게 책정되었음을 고찰하였으며, 이 연구결과 모체의 임신 수유 기간동안의 vitamin B-6 섭취가 탄생과 신생아 기간동안의 건강상태와 영양상태에 밀접한 관계가 있음을 볼 수 있었다.