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Vitamin B-6 Nutritional Status of Breast-fed and Formula-fed Preterm Infants

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ABSTRACT

Concentrations of total vitamin B-6 in human milk as well as individual B-6 vitamers have important implications for the nutritional management of breast-fed(BF) infants. Vitamin B-6 status was assessed in 3 groups of infants : two groups preterm(PT) BF infants whose mothers were supplemented with 2 or 27mg pyridoxine(PN)-HCl ; a sub group of formula-fed(FF) PT infants. Mothers and infants were assessed weekly during the 28-day post feeding. Throughout the neonatal period, levels of total vitamin B-6 and percentages of pyridoxal(PL) in breast milk were lower in PT than T mothers, even in mothers supplemented with 27mg PN-HCl. Total vitamin B-6 levels in PT milk paralleled maternal supplementation but percentage distributions of B-6 vitamers did not change. Vitamin B-6 intakes of BF preterm infants paralleled their mothers' level of supplementation and were markedly lower than intakes of FF infants. Vitamin B-6 inadequacy of infants in the 2mg group was suggested by vitamin status parameters. Vitamin B-6 intakes of PT infants correlated with their plasma pyridoxal-5'-phosphate(PLP) levels and erythrocyte alanine aminotransferase(E-ALAT) activity ; all parameters such as plasma PLP, PL/PLP ratio and stimulation % of E-ALAT were highest for FF PT infants. The positive correlation of vitamin B-6 levels in breast milk with gestational age may contraindicate its adequacy for some PT infants.

KEY WORDS : breast-fed · formula-fed · preterm infant · vitamin B-6 status · vitamin B-6 supplementation · pyridoxal-5'-phosphate.

Introduction

Vitamin B-6 levels in term (T) milk are responded rapidly to changes in maternal intake whereas preterm(PT) milk may be resistant to such changes. Previous studies of T milk show that vitamin B-6 concentration in human milk is influenced by vitamin B-6 intake and nutritional status of the lactating mother[1,2], stage of lactation[1], length of gestation[3] and use of drugs[4]. All of these factors compound the difficulty in establishing a recommended/adequate level of vitamin B-6 in human milk for the breastfed infant.

Lactating women whose intakes of vitamin B-6 are low are at risk of secreting milk which is inadequate in this vitamin for their infants. Information is very limited about the vitamin B-6 composition of PT human milk and its adequacy for the PT infant. The nutritional adequacy of banked mature hu-

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man milk for PT infants has been questioned by findings that it failed to support theoretical intrauterine growth rates of PT infants[5-8]. Unlike the findings with pooled mature human milk, PT infants fed their own mother's milk showed a more rapid rate of growth with respect to length and head circumference and were able to gain weight rapidly[8].

Superimposed on this situation of reduced accumulation of vitamin B-6 in the PT infant is the possibility of an increased vitamin requirement for conditions that typically result from immaturity of various organ system[9]. Comparison of plasma pyridoxal-5'-phosphate(PLP) concentrations and erythrocyte alanine aminotransferase(E-ALAT) activity of PT infants fed formula and those fed their own mothers milk could provide information useful to the vitamin B-6 nutritional management of these infants.

In view of findings that milk of PT mothers may contain low levels of vitamin B-6, and that vitmain B-6 supplementation of PT infants may be necessary, the study was designed to examine the effects of PN-HCl supplementation of PT mothers on the B-6 vitamer levels of their milk. Also, a comparison was made of the B-6 nutritional status of 1) breast-fed (BF) PT infants whose mothers were supplemented with PN-HCl, 2) PT infants who were fed a commercial preterm infant formula.

Materials and Methods

1. Subject selection

Eleven lactating American women, 18yr of age or older, and twenty-one PT infants (28 to 36 weeks gestational age) were recruited to participate in this study. Eleven PT infants were fed their own mother's milk and ten PT infants of the same gestational age were fed a commercial formula. All infants with chromosome or congenital abnormalities were excluded, e.g. cardiac defects, inborn errors of metabolism, small for gestational age, severe respiratory distress syndrome, necrotizing enterocolitis, feeding or gastrointestinal disorders and those on ventilators for more than 7days. Infants of mothers with prenatal complications of diabetes, renal disease, or use of drugs known to interfere with vitamin B-6 metabolism such as isoniazid, hydralazine, penicillamine, cycloserine, and semicarbazide were also not included. All procedures were approved by both the Hospital and the University Committees on the Use of Human Subjects.

2. Experimental design

The experimental period of this study was the first 28 days post feeding. Thirteen lactating mothers who delivered PT infants were assigned by computerized to one of two vitamin B-6 treatment groups. The mothers in one treatment group received daily a commerical prenatal multivitamin-mineral supplement containining 2.0mg PN-HCl(1.7mg PN) ; the other group of 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)[10] for lactating women and available over-the-counter. PT infants received either a commercial formula(Similac Special Care ; Ross Lab.) designed for PT infants or their own mother's milk. This formula was clinically labled SCF20, which contained 2.9mg/L of PN. Laboratory studies were initiated(i.e. "0 time") when at least 75% of the PT infant's enteral caloric intake was achieved. Samples of venous blood were collected from PT infants on 0, 7, 14, 21 and 28 days post feeding. Samples of milk were collected from mothers at each feeding on the same day. Weight and length measurements of infants were obtained weekly during the 28-day study. These measurements were compared with growth reference percentiles of an intrauterine weight curve reference[11] for preterm infants. Maternal nutrient intakes, estimated from foods included in diet records, were calculated by use of

a computer program[12].

3. Estimation of milk intake volume by infants

Test weighing was used in this study as previously described[13]. Daily caloric intakes(kcal/kg/d) were calculated using a mean value of 0.72kcal/mL of milk [14]. For formula-fed PT infants and for PT infants fed their mother's expressed milk, the volume of formula/milk consumed was measured and recorded by a pediatric nurse.

4. Biochemical analysis

Mothers' expressed milk was collected as previously described[13] and stored at −30℃ until analyzed. 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 by venipuncture by a medical technician at the hospital. Blood samples were centrifuged ; plasma and erythrocyte was removed in subdued light and frozen at −30℃ until analysis for B-6 vitamers and PLP levels and alanine aminotransferase activity. Plasma PLP level was measured enzymatically with tyrosine decarboxylase apoenzyme according to the method of Chabner and Livingston[15] as modified by Lumeng and Li[16]. A micro adaptation of the procedure, perfected in our laboratory for the use of 100μL plasma samples[17], was used. The vitamer forms of vitamin B-6 in plasma and milk were measured by the of cation-exchange reverse-phase high-performance liquid chromatography[18-20]. Total protein content of breast milk samples was determined by the method of Lowry et al. using human serum albumin as a standard [21].

5. Statistical analysis

Statistical analysis of data was done in conjunction with the Department of Statistics, Purdue University ; programs for the Statistical Analysis System (SAS) were used[22]. Two-way analysis of variance was done and 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.

Results

1. Milk volume intake of infants

Milk volumes increased with increasing age in all groups during the first month. Levels of maternal PN-HCl supplementation did not significantly($p>0.05$) affect the volume of milk intake in PT infants (Table 1). Volume of milk intake paralleled age in all groups of infants during the first month ; however, as calculated on the basis of body weight(mL/kg/d), milk intake did not change significantly with age.

2. Total vitamin B-6 & protein concentration in PT milk

Throughout the neonatal period, total vitamin B-6 levels in PT milk paralleled the level of maternal supplementation ; levels were significantly lower for mothers supplemented with 2mg PN-HCl/d than for those supplemented with 27mg/d(Fig. 1). In contrast to vitamin B-6 content, protein levels in PT milk declined in all groups with the progression of lactation(from 2.3g/dL to 1.7g/dL).

3. Vitamin B-6 intake of infants

Vitamin B-6 intake of PT infants increased with age during the neonatal period except PT infants of mothers supplemented with 2mg PN-HCl/d(Fig. 2) Vitamin B-6 intakes of breast-fed(BF) PT infants paralleled their mother's level of supplementation. The vitamin intakes of formula-fed PT infants was markedly higher than that of other PT groups.

Vitamin B-6 intakes of most PT infants whose mothers supplemented with 2mg PN-HCl/d did not meet the RDA of vitamin B-6 for infants of 0.3mg/d at 21 and 28days post feeding. When maternal intakes of vitamin B-6 exceeded ten times the RDA for

Table 1. Volume of milk intake of preterm infants

Days of post feeding	Breast-fed		Formula-fed[1]
	Maternal PN-HCl supplement(mg/d)		
	2	27	
Days	Milk intake volume, mL/d		
7	258± 17[2]	342± 80	257± 20
14	372± 54	310± 59	371± 27
21	469± 56	483± 135	456± 43
28	536± 65	611± 158	595± 42
	Milk intake volume, mL/kg/d		
7	148± 15	180± 2	139± 9
14	193± 36	156± 17	168± 5
21	203± 30	182± 46	182± 10
28	222± 33	244± 44	210± 11

1) Infant given daily commercial formula(Similac Special Care) containing 2.9mg/L
2) Mean± SEM

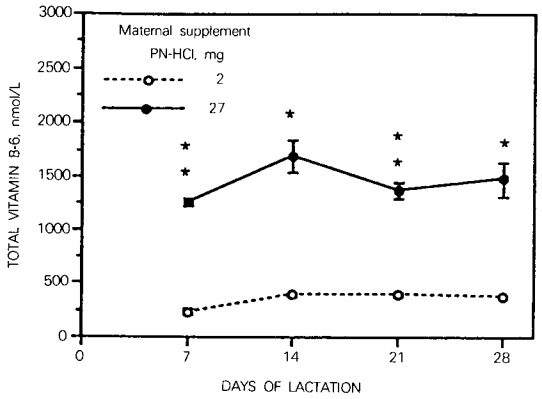

Fig. 1. Vitamin B-6 concentration of preterm milk from mothers supplemented with two different levels of vitamin B-6 during the neonatal period. Vertical bars represent mean± SEM.
* P<0.001, **P<0.0001 significantly different from 2mg supplemented counterpart.

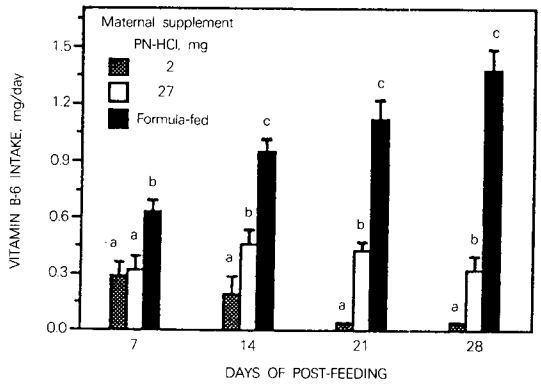

Fig. 2. Vitamin B-6 intakes of preterm infants receiving different feeding treatments during the neonatal period. Vertical bars represent mean± SEM.
[abc] Means of different treatments on the same day without a common letter are significantly different from each other($p<0.05$). The current RDA(1989) of vitamin B-6 intake/day for infants, 0-6 mo age is 0.3mg/d.

lactation, infant intakes of the vitamin meet the RDA during 28days post feeding. Throughout the neonatal period, vitamin B-6 intakes of FF infants exceeded the RDA for young infants.

Mean ratio of vitamin B-6/protein intake(μg/g) of the 2mg group did not meet the recommended ratio of 15μg/g at 21 and 28 days post feeding whereas other groups exceeded this ratio(data not shown). During the last two weeks of the study when the 2mg PN-HCl supplementation group did not receive human milk fortifier(HMF) or multi-vitamin drops, the ratio of vitamin B-6 to protein intake of this group was significantly lower than all other groups.

4. Distribution of B-6 vitamers in PT milk

Mean distribution of five B-6 vitamers in milk, expressed as percent of total vitamin B-6 concentra-

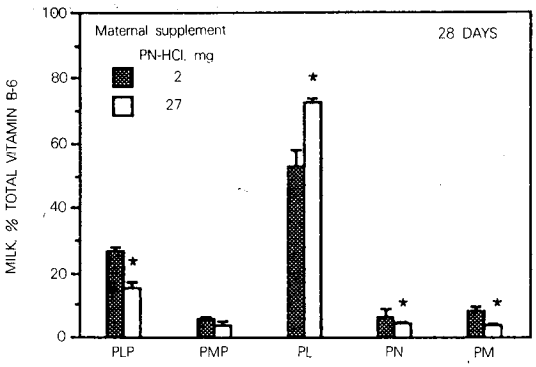

Fig. 3. B-6 vitamers experessed as percent of total vitamin B-6 in preterm milk at 28days of post feeding. Vertical bars represent mean± SEM.
* P<0.01 significantly different from 2mg supplemented counterpart.

tion is shown in Fig. 3. PL was present in the highest percentage among the five vitamers measured in PT milk. Percentage of PL was high in milk of mothers supplemented with 27mg PN-HCl/d than those supplemented with 2mg. Percentages of PMP, PM and PN were similar between two different supplementary groups ; however, PLP was higher in PT milk of the 2mg supplemented group compared to the 27mg/d supplemented group.

5. Vitamin B-6 parameters of PT infants

Vitamin B-6 parameters varied with days of post feeding of infants, type of feeding and level of maternal vitamin B-6 supplementation(Table 2). FF infants had the highest plasma PLP levels. Throughout the neonatal period, breast-fed PT infants of the 2mg supplemented group had the lowest levels.

Mean PLP concentration in cord plasma at delivery(day 0) was 93± 13nmol/L in all PT infants(Table 2). At 7days post feeding, plasma PLP concentrations in all groups of infants were strikingly lower than cord values except FF group. Plasma PLP levels of PT infants during 8 to 28days post feeding correla-

Table 2. Plasma and erythrocyte parameters of vitamin B-6 nutritional status of preterm infants

	Breast-fed		Formula-fed[1]
	Maternal PN-HCl supplement(mg/d)		
Days of post feeding	2	27	
Days		Plasma PLP, nmol/L[2]	
0	95± 5[3]^{a}	103± 6^{a}	87± 9^{a}
7	41± 4^{a}	76± 5^{a}	132± 20^{b}
14	31± 2^{a}	93± 16^{b}	194± 24^{c}
28	23± 3^{a}	78± 12^{b}	190± 27^{c}
		Plasma PL/PLP	
0	0.16± 0.02^{a}	0.57± 0.04^{b}	0.54± 0.10^{b}
7	0.22± 0.03^{a}	1.19± 0.02^{b}	0.92± 0.11^{b}
14	0.34± 0.03^{a}	0.53± 0.03^{b}	0.69± 0.03^{c}
28	0.37± 0.03^{a}	0.43± 0.02^{ab}	0.58± 0.07^{b}
		Stimulation of E-ALAT by PLP, %[4]	
0	8± 2^{ab}	11± 2^{a}	4± 2^{b}
7	4± 2^{a}	4± 2^{a}	4± 1^{a}
14	17± 3^{a}	7± 2^{b}	4± 2^{b}
28	22± 3^{a}	7± 1^{b}	4± 1^{b}

1) Infant given daily commercial formula(Similac Special Care) containing 2.9mg/L PN
2) Determined by HPLC
3) Mean± SEM
4) % stimulation E-ALAT(normal values<16.0 ; high values>160)
^{abc} Means within a row without a common superscript letter are significantly different from each other ($p<0.05$)

ted with their vitamin B-6 intakes($r=0.847$).

High percent stimulation of E-ALAT by PLP(suggesting inadequacy of vitamin B-6) was observed at 14 and 28days of age in infants of PT mothers supplemented with 2mg PN-HCl/d. Similar to plasma and erythrocyte PLP values, percent stimulation of E-ALAT was also correlated with vitamin B-6 intakes of infants(data not shown).

FF infants had the highest plasma PL/PLP ratio ; BF PT infants of the 27mg supplemented group had the next highest levels ; and throughout the neonatal period, BF PT infants from the 2mg supplemented group had the lowest levels.

6. Growth of infants

Growth performance of PT infants was evaluated by percentile of the Lubchenco intrauterine growth curve[11] was used. Birth weight and weight at 28days post feeding, both expressed as percentile were positively related($r=0.519$)(Fig. 4). Most infants increased in weight percentiles during the first 28days ; this change was greater for infants who were in the lower percentiles at birth. Birth weight of infants and gestational age(GA) were significantly positively correlated($r=0.705$)(Fig. 5).

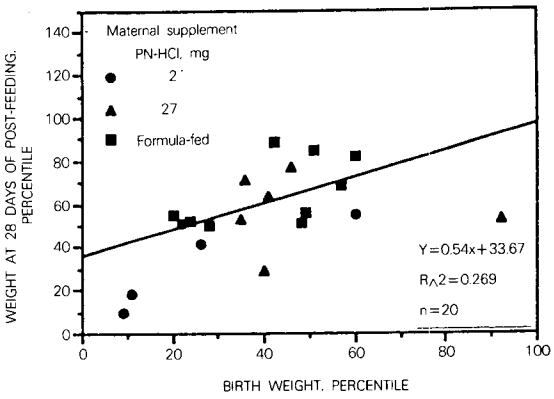

Fig. 4. Relationship of birth weight to weight at 28days of post feeding, both expressed as percentile.

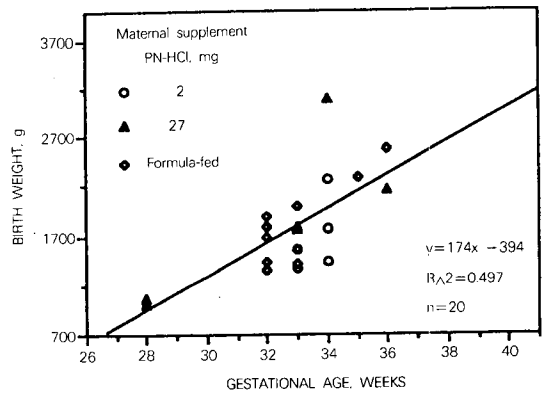

Fig. 5. Relationship of gestational age and birth weight of preterm infants.

Discussion

1. Levels of total vitamin B-6 & protein in PT milk

Total vitamin B-6 concentration of human milk as well as the concentration of individual B-6 vitamers have important implications for the nutritional management of BF infants. Our findings agree with previous reports[3,4,23,24] that vitamin B-6 concentrations in milk are influenced by maternal intake of vitamin B-6 and that PT milk (27−37wk gestation) is significantly lower in total vitamin B-6 levels than T milk.

Low concentrations of total vitamin B-6 concomitant with a smaller percentage of PL observed in PT milk(28−34wk gestation), even in supplemented mothers, could adversely influence the nutrition of PT infants who are fed human milk. PL is the predominant vitamer in milk and most responsive to vitamin B-6 intake. Studies on absorption rates of the individual B-6 vitamers in rats showed that PL has a twofold higher intestinal absorption rate than PN or PM[25,26]. 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.

The higher protein content of PT milk is sufficient to meet the fetal growth requirement for nitrogen. Although the PT milk has a higher protein concent-

ration than T milk of previous report[12], the protein concentration of PT milk declines in a similar fashion during lactation from 2.3 to 1.7g/dL. The reduction in protein concentration may complement the lower protein needs of late infancy. The low level of vitamin B-6 and low ratios of vitamin B-6/protein observed in PT milk could be related to stress, prematurity and reduced maternal stores of the vitamin.

2. Assessment of vitamin B-6 nutritional status of PT infants

Vitamin B-6 intakes of breast-fed infants paralleled their mothers level of supplementation and were markedly lower than intakes of formula-fed PT infants. When maternal intakes of vitamin B-6 met the RDA for lactation, infant intakes did not meet the RDA until 28days of post feeding. In a review of the vitamin B-6 requirements of PT infants, Schanler et al[27] speculated that enteral needs for vitamin B-6 by PT infants[28] may not be met by human milk. Such considerations have led to recommendations that PT infants receiving human milk be supplemented with vitamin B-6.

Biochemical findings of PT infants paralleled their dietary data, e.g., plasma PLP levels and percentage of stimulation of E-ALAT activity by PLP correlated with vitamin B-6 intakes. The higher plasma PLP concentrations of FF preterm infants compared to BF in this study and also in the study of McCoy et al.[29] of preterm BF and FF infants were associated with higher vitamin B-6 intakes by FF infants. High levels of vitamin B-6, normally observed in blood of neonates, have been reported to fall rapidly when the vitamin is supplied in lesser amounts by diet than by maternal blood in utero[30]. Similarly the difference observed in PLP levels in FF and BF infants appeared to be diet-related. In breast milk, the principal form of vitamin B-6 is PL[31] whereas the form in formula is PN-HCl. Vanderslice et al[31], suggested that even though infants appeared to readily assimilate PN, different forms of the vitamin may vary in bioavailability to the infant. Differences in the findings for BF and FF infants in this study may be associated with form of the vitmer fed. Ratien et al.[32] suggested that infants were capable of producing PLP irrespective of the vitamin source.

High stimulation of E-ALAT>16% following the in vitro addition of PLP, an indicator of vitamin B-6 inadequacy, was observed in breast-fed PT infants of mothers supplemented with 2mg PH-HCl/d at 14 and 28dyas of age. Both the dietary and biochemical findings indicated marginal vitamin B-6 nutritional status in this group of infants.

Weekly changes in weight percentile from birth to 28 days were correlated with vitamin B-6 intakes ($r=0.369$). Breast-fed PT infants of mothers supplemented with 2mg PN-HCl/d had the smallest weekly changes in weight percentile. The results of this study showed a growth advantage for breast-fed infants when the maternal supplement was 27mg PN-HCl/d compared to 2mg/d. In the case of PT infants, the 27mg maternal supplement or formula offered a growth advantage and supported rates of weight gain comparable to those observed during the last trimester of intrauterine life ; however, supplement levels between 2 and 27mg were not assessed.

3. Nutritional adequacy of milk for PT infants

The nutritional adequacy of banked mature human milk for PT infants has been questioned by findings that it failed to support theoretical intrauterine growth rates of PT infants[5-8]. Pasteurization, necessary for pooled milks, alters the immune and perhaps other factors in the milk[33,34]. The low protein concentration of pooled T human milk is probably the major cause of the poor growth. Unlike the findings with pooled mature human milk, PT ifants fed their own mother's milk showed a more rapid rate of growth and were able to gain weight rapidly[8]. The higher protein and total N content[27,35], as well as increased levels of some electrolytes and possibly of fat and energy suggest that PT milk may be a

more appropriate source of nutrition for the PT infant than T milk[36-39]. Recently Chan et al.[40] reported that there is little effect on the growth or behavioral or protein status of PT intants who fed either human milk or formulas designed for T or PT infants. However, Lucas et al.[41] suggested that donor milk feeding was associated with advantages for later development PT infant that may have offset any deleterious effects of its low nutrient content for PT infants.

There are uncertainties whether very immature infants are best nourished with their own mother's milk or with a specially designed formula. Formula-fed infants are known to gain weight faster than those fed breast milk[42]. McCoy's findings[43] illustrate that current IV alimentation programs and PT special formula provide high amounts of vitamin B-6 for PT infants. In this study, vitamin B-6 intakes of formula-fed PT infants was highest(ranging from 0.58－1.53mg/d) among the six groups, all of which exceeded the RDA. The positive correlation of vitamin B-6 levels in breast milk with gestational age may contraindicate its adequacy for some PT infants, since their plasma PLP levels may already be low owing to the lack of accumulation of the vitamin during the last weeks of gestation.

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= 국문초록 =

모체의 비타민 B-6 섭취 상태가 조산아의 비타민 B-6 영양상태에 미치는 영향

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모유의 total 비타민 B-6와 B-6 vitamers의 농도측정은 모유영양아의 영양관리를 위해서 매우 중요한 요소이다. 본 연구는 수유방법과 시기, 비타민 투여량이 조산아의 비타민 B-6 영양상태에 미치는 영향에 대해 연구하였다. 영양상태 평가는 조산아를 대상으로 세군으로 나누어 수유부를 2mg과 27mg PN-HCl 섭취하는 두군으로 나누고 한군은 조제유를 섭취하였다. 신생아의 비타민 B-6섭취량을 수유이후 1달동안(신생아기) 1주 마다 측정하였고 영양상태 평가를 위해서 출생직후 부터 신생아기동안 매주 혈액내 비타민 B-6 영양상태 평가지표를 이용해서 측정하였다.

모유의 total vitamin B-6 농도와 pyridoxal(PL)의 분포가 2mg의 PN-HCl/d 섭취한 군보다 27mg의 PN-HCl/d 섭취한 군에서 높게 나타난 것으로 보아 비타민 B-6 섭취상태가 모유속에 함유된 비타민 B-6 함량에 유의적으로 영향을 미침을 알 수 있었다. 모유영양아의 비타민 B-6 섭취량은 모체의 비타민 섭취량에 비례하였고 조제영양아의 섭취량보다 유의적으로 낮게 나타났다. 모체가 2mg 섭취한 군의 조산아들의 영양상태가 불충분하다는 결과는 혈액의 지표(plasma PLP, PL/PLP ratio, stimulation % of E-ALAT)와 성장도(weight percentile)로써 판단할 수 있었다. 조제영양아의 혈액내 지표농도가 모유아보다 높게 나타남은 섭취된 vitamer의 형태가 다름과 관련이 있었다. 이 연구 결과로 모체의 수유기간 동안의 비타민 B-6섭취가 신생아기간동안 영양상태에 영향을 미치는 것으로 보인다. 조산아들이 정상적인 성장을 하고 바람직한 영양상태를 유지할 수 있게하기 위해서 조숙유(preterm milk)를 섭취해야 하므로 수유부와 조산아의 비타민 B-6 영양상태 개선을 위해서 수유부의 비타민 B-6 첨가를 권장하고자 한다.