

## B-6 Vitamers and $\beta$ -Glucoside Conjugates in Milk of American and Egyptian Women during the First Six Months of Lactation

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### ABSTRACT

Levels and distribution of five B-6 vitamers(PMP, PM, PLP, PL, and PN) and pyridoxine  $\beta$ -glucoside conjugates(PN-glucoside) were examined in milk of American women who received supplements of 2.5 or 10mg PN HCl/d and of unsupplemented Egyptian women during the first six months of lactation. B-6 vitamer and PN-glucoside levels in human milk were determined by reverse-phase HPLC. Pyridoxal(PL), which has been reported to be the most rapidly absorbed form of vitamin B-6 and may facilitate bioavailability, was the predominant vitamer in human milk of all three groups. Pyridoxal made up 72% of total vitamin B-6 for the 2.5mg supplemented group, 76% for the 10mg group, and 59% for the Egyptian group. Level and percent PL were significantly lower for Egyptian women. Mean growth of the two American groups was similar to each other and within the normal range of the NCHS reference, however, Egyptian infants showed growth faltering at 6 months. The percent of PN-glucoside, a less bioavailable form of vitamin B-6 in humans was 1% in milk of American women and was 11% in Egyptian women and these values were significantly different. For Egyptian women, total vitamin B-6 levels in breast milk correlated positively with animal protein intake( $r=0.91$ ) and percent PN-glucosides( $r=0.53$ ) and negatively with plant protein intake( $r=-0.55$ ). These findings showed that high plant protein intake was associated with low concentrations of PL and total vitamin B-6 in human milk. (*Korean J Nutrition* 30(4) : 425~433, 1997)

**KEY WORDS** : breast milk · vitamin B-6 · Pyridoxine-glucoside · bioavailability.

### Introduction

One of the areas of uncertainty in vitamin B-6 nutrition is bioavailability, defined as the proportion of ingested vitamin absorbed and utilized in the body. Metabolic studies of vitamin B-6 have provided ample evidence that it is advantageous, even necessary to determine the concentrations of all forms of vitamin B-6.

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Studies on the absorption rates of individual B-6 vitamers in vascularly perfused small intestine of rats indicated that pyridoxal(PL) was absorbed twice as fast as pyridoxamine(PM) or pyridoxine(PN) by passive diffusion<sup>1)</sup>. This finding agreed with that of another study<sup>2)</sup> in which the relative rates of vitamer absorption were  $PL > PM > PN$ .

Recent findings<sup>3)</sup> showed that even though vitamin B-6 intakes were higher for formula fed(FF) infants than breastfed(BF), growth was within the normal range and was not significantly different for these two

groups of infants. The major form of vitamin B-6 in formula is PN whereas in human milk, PL is the major B-6 vitamer. This suggested that the level of PN in formula could be excessive for infants or not well-absorbed whereas in human milk the high proportion of PL possibly facilitates bioavailability. An even larger percentage of vitamin B-6 in human milk is absorbed as PL than would be indicated, since pyridoxal phosphate (PLP) is dephosphorylated at the brush border of the intestinal cell and absorbed as PL.

Hamaker et al.<sup>4</sup> reported that the percentage of PL was approximately 25% lower in milk of unsupplemented women compared to supplemented. These investigators observed that milk which contained the highest level of total vitamin B-6 had proportionally more PL than milk which contained less total vitamin.

The possible nutritional significance of conjugated forms of vitamin B-6 has been the subject of intense interest following the isolation and identification of vitamin B-6 conjugates from rice bran<sup>5</sup>. Kabir et al.<sup>6</sup> found a glycosylated form of vitamin B-6 in a variety of plant foods but noted its absence in animal foods. These workers suggested that  $\beta$ -glucoside conjugates forms of vitamin B-6 were not biologically available to humans. The commonly used microbiological assay<sup>7</sup> measures the free forms of the vitamin after the conjugated linkages are hydrolyzed by acid or enzymatic methods. In this regard, the recently developed HPLC method<sup>8</sup> for the determination of B-6 vitamers is advantageous because the extraction techniques used in these procedures involve deproteinization by agents that do not hydrolyze the  $\beta$ -glucosidic linkage of conjugated forms of the vitamin. It is important that vitamin B-6 conjugates be more completely evaluated in order that the nutritional quality of diets, in particular diets of infants, containing these forms of the vitamin can be more accurately assessed.

The objectives of this study were to 1) compare the relative distribution of B-6 vitamers in milk of supplemented American women and unsupplemented Egyptian women during the first 6 months of lactation ; 2) estimate the amount of  $\beta$ -glucosides conjugated with vitamin B-6 in the milk of these women and 3) determine whether the  $\beta$ -glucoside levels in human milk were associated with plant protein intake.

## Materials and Methods

### 1. Subject selection and milk sample collection

American volunteers were recruited a few weeks prior to their delivery. They were supplemented with 2.5 or 10mg PN HCl/d and milk samples were obtained monthly during the first 6 months of lactation. Mothers expressed 5–10ml samples of breast milk at each feeding during a 24 hr period. Milk samples were protected from light by use of amber-colored vials and were frozen immediately after collection.

Milk samples were also collected monthly during the first 6 months of lactation from 38 Egyptian women participating in a large human nutrition study supported by the U.S. Agency for International Development. Milk samples were frozen and later shipped in dry ice by air to the Nutrition Laboratory at Purdue university.

### 2. Microbiological analyses of vitamin B-6

Total vitamin B-6 content in human milk was determined by a microbiological assay that employed *Saccharomyces uvarum* and was based upon the methods of Storvick et al.<sup>9</sup>, Brin and Thiele<sup>10</sup>, and Toepfer and Polansky<sup>7</sup>. Samples were hydrolyzed by 0.055 N HCl and autoclaving for 5hr at 15psi. Standard solutions of PN HCl(1, 2, 4 and 6ng) were also incubated with a drop of the culture of *S. uvarum* at 32°C for 22hr in a shaking water bath. Turbidity was measured at 550nm on a Spectrophotometer. Concentrations of total vitamin B-6 in the milk samples were calculated by use of a standard curve.

### 3. Measurement of the glycosylated form of vitamin B-6

#### 1) $\beta$ -d-glucosidase enzyme method

Glycosylated vitamin B-6 concentration of milk was measured by a procedure adapted from Kabir et al.<sup>11</sup>. In subdued light, milk samples were mixed with 0.1M phosphate buffer and thymol and the mixture was titrated to pH 5.0 with 1 N HCl, and then 60 units of  $\beta$ -d-glucosidase was added. Samples were then incubated for 2 hr at 37°C in a water bath. After titrating with 6 N KOH and filtering, samples were

then treated as filtrate for microbiological analysis.

## 2) Pyridoxine-glucoside standard method

Alfalfa seeds were soaked in water overnight, washed and drained. PN HCl was added in a small aliquot of water daily to seeds. After a 5 day period in the dark at ambient temperature (22°C), the resulting sprouts were washed to remove any excess PN HCl. After purification, PN-glucoside (PNG) was isolated using cation-exchange chromatography and PNG fractions were monitored spectrophotometrically at 325nm. This procedure was followed by reverse-phase HPLC as described later in this section. The pooled fractions containing pure PNG were then lyophilized and diluted appropriately for quantification by the same HPLC method<sup>12)</sup>.

## 4. HPLC Analysis

The reverse-phase HPLC system consisted of a Constametric II-G pump, Rheodyne injector, and Guard column. The detector was a Shimadzu fluorescence spectromonitor set at 330 nm for excitation and 400 nm for emission. The post column syringe pump delivered 8ml bisulfite solution/hr. Mobile phase consisted of 0.033M KH<sub>2</sub>PO<sub>4</sub> buffer containing 3% methanol adjusted to pH 2.9 with orthophosphoric acid.

Standards (concentrations in nmol/L : PMP, 40 ; PM, 60 ; PLP, 80 ; PL, 246 ; PN, 120) were prepared from concentrated frozen stock solutions by dilution with the mobile phase buffer. Milk samples were diluted with an equal volume of mobile phase buffer and centrifuged at 4°C. The supernatant was removed and then diluted again with an equal volume of buffer.

## 5. Assessment of Infant Growth

Weight measurements of infants were determined

monthly during the first 6 months of age by use of a K-tron electronic balance which was accurate to 1g. These measurements were compared with weight reference percentiles published by the National Center for Health Statistics (NCHS)<sup>13)</sup>.

## 6. Statistical analysis

Analyses were carried out with the use of the SAS Statistical package<sup>14)</sup>. Most analyses were based on the General Linear Model (PROC GLM) which includes simple and multiple regression, analysis of variance and covariance. Significant differences between means were tested using Student's T-test<sup>15)</sup> and Student-Newman-Keul (SNK) test<sup>16)</sup> and multiple regression was determined.

## Results

Descriptive information regarding the American and Egyptian women who participated in this study is presented in Table 1. This data indicated that mean weight for height for the two groups was average according to the BMI reference of the Second National Health and Nutrition Examination survey 976-80 (NHANESII)<sup>17)18)</sup>. However, BMI data for individuals showed that eleven Egyptian were overweight (BMI > 27.3) as contrasted with one American woman.

### 1. B-6 vitamer concentration in milk

Mean concentrations of five B-6 vitamers in milk from American and Egyptian women are shown in Table 2. Concentrations of PLP, PL, and PN in milk were significantly different among groups whereas PMP and PM were not significantly different (Fig. 1). PL was the predominant B-6 vitamer in all groups and ranged up to 1593 nmol/L in the 10mg PN

**Table 1.** General description of mothers

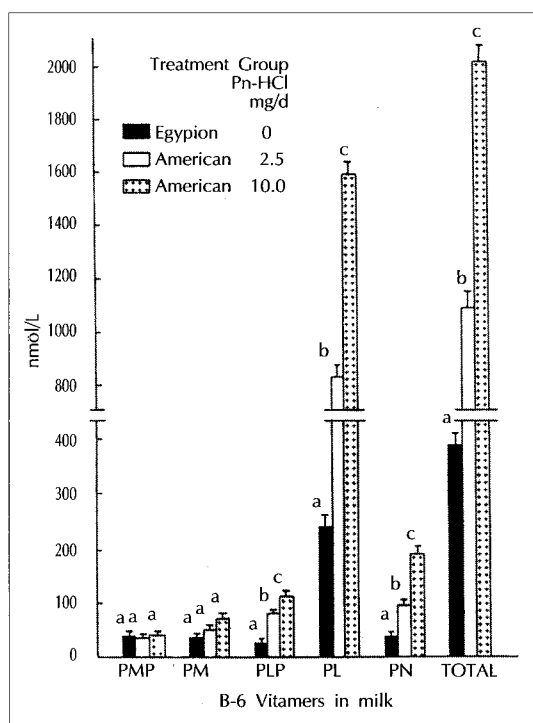
Treatment Group	Number of Subjects	Age(years)	Parity	Height(cm)	Weight(kg)	BMI <sup>1)</sup> (kg/m <sup>2</sup> )
American	12	29.2 ± 1.5 <sup>2A)</sup> (22 - 37) <sup>3)</sup>	1.4 ± 0.6 <sup>A)</sup> (0 - 5)	163.0 ± 2.7 <sup>A)</sup> (155 - 178)	58.4 ± 2.3 <sup>A)</sup> (50 - 71)	22.0 ± 1.0 (20 - 29)
Egyptian	38	27.6 ± 1.1 <sup>A)</sup> (17 - 45)	4.6 ± 0.4 <sup>B)</sup> (0 - 9)	154.5 ± 2.5 <sup>B)</sup> (146 - 167)	63.3 ± 0.9 <sup>A)</sup> (46 - 85)	25.8 ± 0.9 (20 - 35)

1) Body Mass Index = Weight(kg)/Height(m<sup>2</sup>)

2) Mean ± SE

3) Range

A, B Means within a column which do not have a common superscript letter are significantly different from each other (p < 0.05)



**Fig. 1.** Relationship among concentrations of B-6 vitamers in human milk. <sup>abc</sup>Means without a common letter are significantly different from each other ( $p < 0.05$ ).

HCl/d group. Total vitamin B-6 concentrations in three groups were significantly different ( $P < 0.05$ ) from each other.

## 2. Distribution of B-6 vitamers in breast milk

Mean distribution of B-6 vitamers in breast milk, expressed as percent of total vitamin B-6 concentration, is presented in Table 3. For all groups of women, PL was the highest among five vitamers in breast milk (71.6% for the 2.5mg PN HCl/d supplemented group, 76.3% for 10mg group, and 58.7% for the Egyptian group). Means were significantly ( $P < 0.05$ ) different among the three groups. In the American groups, percentages of PLP and PN were the next highest values to PL whereas in the Egyptian group, percentages of PMP, PM, PLP, and PN were similar (Table 3).

## 3. Comparison of microbiological and HPLC method

Mean total vitamin B-6 concentration in milk determined by HPLC was 5.2% lower than that determined by microbiological assay using *S. uvarum*. However, the difference was not statistically significant. A correlation coefficient of vitamin B-6 concentration

**Table 2.** B-6 vitamin concentration from vitamin B-6 supplemented American women and unsupplemented Egyptian women during the first 6 months of lactation

Treatment Group	N <sup>1)</sup>	B-6 Vitamers(nmol/L)					
		PMP	PM	PLP	PL	PN	Total
American							
PN HCl							
2.5mg/d	4(46) <sup>2)</sup>	37±7.12 <sup>A</sup>	51± 9.3 <sup>A</sup>	81± 8.7 <sup>A</sup>	822±57.6 <sup>A</sup>	98±11.7 <sup>A</sup>	1090±66.1 <sup>A</sup>
10mg/d	8(94)	43±6.8 <sup>A</sup>	72±11.8 <sup>A</sup>	113±11.7 <sup>B</sup>	1593±56.4 <sup>B</sup>	191±15.4 <sup>B</sup>	2012±63.3 <sup>B</sup>
Egyptian	38(76)	41±5.9 <sup>A</sup>	39± 5.5 <sup>A</sup>	27± 4.0 <sup>C</sup>	242±22.8 <sup>C</sup>	39±8.3 <sup>C</sup>	387±34.8 <sup>C</sup>

1) Number of subjects

2) Number of observation

3) Mean ± SE

A, B, C means within a column without a common superscript letter are significantly different from each other

**Table 3.** Percent distribution of B-6 vitamers from vitamin B-6 supplemented American women and unsupplemented Egyptian women during the first 6 months of lactation

Treatment Group	N <sup>(1)</sup>	B-6 Vitamers(nmol/L)				
		PMP	PM	PLP	PL	PN
American		% of Total Vitamin B-6 Concentration				
PN HCl						
2.5mg/d	4(46) <sup>(2)</sup>	4.6±0.7 <sup>3A</sup>	4.7±0.8 <sup>A</sup>	10.6±1.0 <sup>A</sup>	71.6±1.2 <sup>A</sup>	8.7±8.8 <sup>A</sup>
10mg/d	8(94)	2.9±0.4 <sup>A</sup>	3.5±0.5 <sup>A</sup>	8.1±0.8 <sup>A</sup>	76.3±1.0 <sup>B</sup>	9.1±1.6 <sup>A</sup>
Egyptian	38(76)	12.4±1.0 <sup>B</sup>	10.1±1.1 <sup>B</sup>	10.7±1.2 <sup>A</sup>	58.7±1.1 <sup>C</sup>	8.3±1.0 <sup>A</sup>

1) Number of subjects

2) Number of observation

3) Mean ± SE

A, B, C Means within a column without a common superscript letter are significantly different from each other

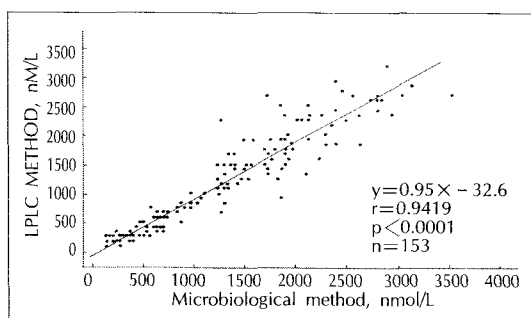


Fig. 2. Correlation of the HPLC and microbiological methods for the determination of total vitamin B-6 concentration in human milk.

determined by two methods was 0.9419, indicating good reliability between the two methods (Fig. 2).

#### 4. Growth of infants

All American infants had weights > 25th percentile of NCHS reference. However, among the 38 Egyptian infants, 16% at birth, 18% at 1 month, 21% at 3 months, and 45% of infants at 6 months of age had weights < 25th NCHS percentile.

Growth velocity of infants, expressed as percent of monthly increment of weight, tended to decrease with age and was generally similar for the three groups of infants. However, after 6 months of age the group supplemented with 10mg PN HCl/d showed statistically higher growth velocity than the other groups. Growth faltering was evident in the Egyptian group. Doubling of birthweight occurred at 4.4 and 5.3 months for American and Egyptian infants, respectively. However, these values were not statistically different.

#### 5. Percent of conjugated pyridoxine-glucoside in human milk

Conjugated PNG in milk was analyzed by two dif-

ferent methods, enzymatic and HPLC. Mean conjugated PNG expressed as percent of total vitamin B-6 is presented in Table 4. In the American group, 1.9% and 0.9% of the PN was glycosylated in milk analyzed by enzymatic and HPLC methods, respectively, and these values were significantly correlated. In the Egyptian group, 15.1% and 10.9% PNG were analyzed by the two methods. Mean percentages of PNG in milk of Egyptian and American women were significantly different.

#### 6. Dietary protein intake and relationship to vitamin B-6 concentration

Americans had a higher consumption of protein from animal sources than the Egyptian group. In contrast, Egyptians had a higher mean protein intake from plant sources than the American group. The relationship of total vitamin B-6 concentration in milk to dietary protein intake was examined. These variables were not significantly related in American subjects but were statistically related in the Egyptian group. Vitamin B-6 concentration in milk of Egyptian women was significantly and positively correlated with their animal protein intake ( $r=0.768$ ). Interestingly, however, the vitamin concentration in Egyptian milk was significantly and negatively correlated with plant source protein ( $r=-0.552$ ,  $P<0.04$ ).

### Discussion

#### 1. B-6 vitamers in HPLC system

A reverse-phase HPLC method was found to be a direct and reliable means of determining the concentrations of five B-6 vitamers (PMP, PM, PLP, PL, PN) in human milk. The fluorescence response was

Table 4. Determination of conjugated pyridoxine-Glucoside as percent of total vitamin B-6 in human milk by two different methods

Treatment		Methods			
Group	N <sup>1)</sup>	Enzymatic	HPLC	R <sup>2)</sup>	P
Conjugated PN-Glucoside(% of Total Vitamin B-6)					
American	16	1.9±1.3 <sup>3A)</sup> 0-4.3 <sup>4)</sup>	0.9±0.1 <sup>A)</sup> 0-2	0.764	<0.0001
Egyptian	14	15.1±1.4 <sup>B)</sup> 1.8-26.4	10.9±0.9 <sup>B)</sup> 2-16.9	0.601	<0.001

1) Number of subjects

3) Mean±SE

2) Correlation coefficient

4) Range

A, B Means within a column without a common superscript letter are significantly different from each other; means within a row were not significantly different

linear for all concentrations of B-6 vitamers found in human milk. There was no significant difference between total vitamin B-6 levels determined by microbiological assay and HPLC method. A high correlation (Fig. 2) was observed between the two methods indicating good comparability of the HPLC and microbiological method for vitamin B-6 analysis. Since the microbiological method includes a hydrolysis step, the total vitamin B-6 concentration from this method contains the glycosylated B-6 vitamer that is known to be less bioavailable<sup>6</sup>. Therefore, values determined by this method may be overestimated. Since the HPLC method enables one to ascertain reliably the various forms of vitamin B-6 and PNG in human milk, it may be particularly useful in estimating the forms which are available. Presently such data for human infants is scanty indeed.

## 2. Concentration and distribution of B-6 vitamers in milk

Total vitamin B-6 concentration in milk, determined by HPLC, was significantly different among the three groups. Vitamin B-6 concentration in human milk is dependent upon maternal intake of the vitamin and hence varies widely<sup>19</sup>. It may be more meaningful to compare percent distribution of B-6 vitamers rather than mean concentrations. PL was the predominant B-6 vitamer in milk of all three groups and values compared well with reports from two ion-exchange HPLC methods<sup>20,21</sup>. However, Egyptian women had significantly lower vitamin B-6 concentration and percentage of PL of total vitamin B-6 than the American groups. The high proportion of PL in human milk may be beneficial to some infants, particularly preterm infants, because it has been reported to be more rapidly absorbed than other forms of vitamin B-6<sup>1,22</sup>. Furthermore, a larger percentage of vitamin B-6 in milk is absorbed as PL than is indicated by PL per se since PLP is dephosphorylated at the brush border of intestine<sup>22</sup> is then absorbed as PL. The mechanism of transport of vitamin B-6 from the circulatory system into the mammary gland is not well established. Spector<sup>23</sup> and Lumeng<sup>24</sup> reported that the phosphorylated forms of the vitamin most readily crossed physiological barriers and/or cell membranes. Predominance of PL in human milk suggests that a dephosphorylating

mechanism is present at the blood : mammary gland barrier.

## 3. Growth of infants during the first 6 months of age

Growth of infants according to the NCHS reference showed that infants whose mothers received supplements of 2.5mg PN HCl/d had the highest birthweight among the three groups of infants studied. Growth velocity was significantly higher for infants whose mothers consumed 10mg PN HCl/d from delivery to 6 months postpartum compared to the other groups. Maternal vitamin B-6 supplementation with 10mg PN HCl/d contributed to the total vitamin B-6 intake of the infants and may have been associated with some catch-up growth observed at 6 months of age.

Egyptian infants who were comparable in mean birthweight to the 10mg group should show less catch-up growth during the 6 month period.

The age of birthweight doubling was significantly correlated to infant birthweight ( $r=0.44$ ,  $p<0.0025$ ) and was not significantly different in two American groups. The infants who failed to double their birthweight by 6 months of age had birthweights of more than 4kg in American groups and 3.2 kg in Egyptian group. These findings agreed with the belief that the age of birthweight doubling is controlled primarily by the size of the infant at birth<sup>3,25</sup>.

## 4. Percentage of conjugated Pyridoxine-glucoside in milk

Percent composition of glycosylated form of vitamin B-6 determined by the microbiological assay and the HPLC method<sup>12</sup> was significantly correlated. The HPLC method was rapid, simple, and reliable ; the values obtained by this method were used in this study.

Glycosylated vitamin B-6 has been shown to be of lower bioavailability than nonglycosylated forms of the vitamin in controlled studies of humans<sup>6</sup> and rats<sup>26</sup>. However, the impact of dietary glycosylated vitamin B-6 upon the vitamin B-6 status of the human is unknown.

Andon et al.<sup>27</sup> assessed whether the dietary intake of glycosylated vitamin B-6 had a significant effect

upon the vitamin B-6 status of lactating women and their infants. They assumed that the fraction of dietary total vitamin B-6 that is present as glycosylated vitamin B-6 is of low bioavailability and that the dietary intake of glycosylated vitamin B-6 comprised a large enough proportion of the total dietary vitamin B-6 content to affect biochemical indices of vitamin B-6 nutritional status. They found that there is no demonstrable effect of dietary intake of glycosylated vitamin B-6 or the glycosylated vitamin B-6 in breast milk on the biochemical indices of vitamin B-6 nutritional status that were measured.

Andon et al.<sup>27)</sup> reported that glycosylated vitamin B-6 comprised a mean 2.5% of the total vitamin B-6 concentration measured microbiologically in breast milk of American women. Also, Reynolds et al.<sup>28)29)</sup> reported that glycosylated vitamin B-6 accounted for a mean 15.3% of the total vitamin B-6 content of breast milk samples collected from 26 Nepalese vegetarian lactating women. The reason for the higher concentration of glycosylated vitamin B-6 in the breast milk of the Nepalese women compared with American women was unclear.

##### 5. Relationship of vitamin B-6 concentration, dietary protein intake and percent of conjugated pyridoxine-glucoside in milk

Egyptian women consumed significantly higher amounts of foods of plant origin (beans, peas and rice) than of animal origin (chicken, lamb, and cheese). Total vitamin B-6 levels in the milk of Egyptian women were correlated negatively ( $p < 0.04$ ) with plant protein intake and plant products are known to contain a high percentage of glycosylated vitamin B-6. Vitamin B-6 is highly glycosylated in rice, navy beans, and soy beans, the major foods of plant origin consumed : 14, 42, and 57% of total vitamin B-6, respectively<sup>11)</sup>. In contrast chicken and lamb, the major foods of animal origin consumed, are good sources of vitamin B-6 with undetectable amounts of PNG<sup>11)</sup>. Animal source foods were highly correlated with vitamin B-6 levels in contrast with plant foods that were negatively correlated. Even though no significant relationship was found between PNG in milk and plant protein intake, a statistical trend ( $r = 0.48$ ,  $p > 0.08$ ) was found. Reynolds et al.<sup>28)29)</sup> observed that the concentration of

plasma PLP in Nepalese infants whose mothers were vegetarians was substantially lower than that of American breastfed infants even though total vitamin B-6 concentrations in breast milk were not significantly different.

In the present study, evaluation was not made of the vitamin B-6 nutritional status of the Egyptian infants who consumed a high percentage of PNG in milk. Such an assessment would possibly have reflected whether the PNG in breast milk of Egyptian mothers was available to the infants.

## Summary and Conclusion

Five B-6 vitamers (PMP, PM, PLP, PL, and PN) and glycosylated vitamin B-6 levels were examined in milk of American women who received supplements of 2.5 or 10mg PN HCl/d and of unsupplemented Egyptian women during the first six months of lactation.

PL was the predominant vitamer in human milk of all three groups representing 72% of total vitamin B-6 for the 2.5mg supplemented group, 76% for the 10mg group, and 59% for the Egyptian group. PL is purported to be the vitamer which is most rapidly absorbed ; the high percentage of this vitamer in human milk may facilitate bioavailability. Mean growth of the two groups of American infants during the first 6 months of age was similar and within the normal range of the NCHS reference. Compared to the American groups, however, 45% of the Egyptian infants were below the 25th percentile at 6 months, indicating growth faltering in these infants.

The percent of glycosylated vitamin B-6 in milk of American and Egyptian groups was 1% and 11%, respectively ; the percentages were significantly different. Glycosylated vitamin B-6 has been reported to be of low bioavailability in humans. Therefore, bioavailability of the high  $\beta$ -glucoside levels in milk of Egyptian women and the effects of this on the vitamin B-6 status of solely breastfed Egyptian infants warrants investigation.

The levels of total vitamin B-6 observed in milk of Egyptian women correlated positively ( $r = 0.77$ ,  $p < 0.001$ ) with animal protein intake and negatively ( $r = -0.55$ ,  $p < 0.04$ ) with plant protein intake. Even though

vitamin B-6 concentration was correlated with conjugated PN-glucoside in milk by linear regression, multiple regression showed that the relationship was a reflection of the high correlation between animal protein intake and vitamin B-6 concentration in milk. There was a tendency ( $r=0.48$ ,  $p>0.08$ ) for percent PN-glucosides in milk and plant protein intake to be related. In Egyptian women, increased plant protein intake was associated with lower vitamin B-6 concentration in breast milk.

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