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Low Vitamin B_6 Status Associated with Slow Growth in Healthy Breast-Fed Infants

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ABSTRACT

To evaluate the effect of vitamin B_6 status on infant growth, we studied longitudinally anthropometry and the erythrocyte parameters that reflect long-term vitamin B_6 status [erythrocyte pyridoxal 5′-phosphate concentration (EPLP), erythrocyte aspartate transaminase basal activity ($EAST_o$), and its activation coefficient (αEAST)] in 44 infants. The infants were exclusively breast-fed for 6 mo, given additional solids according a uniform schedule from 6–9 mo, and formula after 9 mo, if needed. In seven of these infants, a low vitamin B_6 status (EPLP < 10th, and $EAST_o$ > 10th or αEAST > 90th percentile for these values in reference infants) was observed between 4 and 6 mo of age. These seven infants showed slower length velocity (0.30 ± 0.05 *versus* 0.40 ± 0.02 mm/d, $p \leq 0.02$) and deeper fall in length-for-age (−0.69 ± 0.20 *versus* −0.25 ± 0.07 SD score, $p \leq 0.03$) from 6 to 9 mo of age than the similarly fed infants with higher vitamin B_6 status. Preceding vitamin B_6 status remained a significant explanatory factor for length velocity and change in length-for-age in addition to preceding and concomitant weight velocity, when sex, birth size, preceding length gain, and mid-parent height were taken into account. Change in weight-for-age alone explained 16% and 18% and, together with vitamin B_6 status, 23 and 27% of the variation in length velocity and in change in length-for-age, respectively. Thus, in healthy breast-fed infants, according to our results, low vitamin B_6 status is associated with reversibly reduced gain in length. (***Pediatr Res* 38: 740–746, 1995**)

Abbreviations

PPLP, plasma pyridoxal 5′-phosphate concentration
EPLP, erythrocyte pyridoxal 5′-phosphate concentration
EAST, erythrocyte aspartate aminotransferase (EC 2.6.1.1)
$EAST_o$, EAST basal activity
$EAST_+$, EAST stimulated activity
αEAST, EAST activation coefficient = $EAST_+$:$EAST_o$
PN·HCl, pyridoxine hydrochloride
adeqB_6 infants, infants with adequate vitamin B_6 status during breast feeding
lowB_6 infants, infants with low vitamin B_6 status during breast feeding
SDS, SD score

Infant growth patterns are influenced by feeding practice (1–5) in addition to sex, birth size (6), and genetic background (7). After the first 2 mo, breast-fed infants grow slower than formula-fed infants even in well-nourished populations (1–5). It has been discussed whether this growth pattern is physiologic or reflects inadequacies of breast milk. Differences in milk volume (8), and intake of energy, protein (9, 10), and dietary nutrients like iron and zinc that covary with protein (11, 12), have been suggested to explain the difference in growth between breast- and formula-fed infants.

In animals, vitamin B_6 deficiency leads to slowing of weight gain (13, 14), which can be restored by pyridoxine administration (14, 15). The higher the protein intake, the earlier and the greater the growth retardation (15, 16). In human infants, some investigators have also found an association between vitamin B_6 status and growth (17, 18). Snyderman et al observed failure to gain weight in infants on diet devoid of vitamin B_6 (17). In a study by Kang-Yoon et al the breast-fed infants with considerable maternal and/or infant vitamin B_6 supplementation showed greater increase in weight and length *Z* scores than the infants whose mothers were less supplemented (18). In addition, infant vitamin B_6 intake and plasma pyridoxal 5′-phosphate concentration predicted weight gain during the first month of life (18).

Erythrocyte vitamin B_6 parameters, including EPLP, $EAST_o$, and its activation coefficient αEAST, reflect long-term vitamin status. We have reported strikingly higher vitamin B_6 status in formula-fed than in exclusively breast-fed infants during the first 6 mo of life (19). Of formula-fed infants, 50–96% had values for the long-term vitamin B_6 parameters above the 95th percentile for these values in breast-fed infants (19). In addition, we have found that the infant's risk of low vitamin B_6 status increases after 6 mo of exclusive breast

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To follow possible associations between vitamin B_6 status and growth in breast-fed infants fed according to the current WHO feeding recommendations, we used anthropometry and erythrocyte parameters which reflect long-term vitamin B_6 status.

METHODS

Subjects. We followed the growth and nutritional status of 198 infants from birth to 12 mo of age. They were healthy full-term (37–42 gestation wk) singletons with uncomplicated delivery after normal pregnancy, birth weights appropriate for gestational age, and Apgar scores ≥8 at 1 min. Of these infants, the 44 included in this study (20 boys and 24 girls) were exclusively breast-fed for 6 mo, given additional solids according a uniform schedule from 6–9 mo, and formula after 9 mo, if needed. At 12 mo, all the infants were still partly breast-fed; 20 of them were breast-fed and given additional solids, 24 of them were breast-fed and given additional formula and solids.

Vitamin B6 supplementation. At delivery, 30 of the 44 mothers reported that they had taken vitamin B_6 supplementation during pregnancy. The median dose of pyridoxine hydrochloride (PN·HCl) supplement had been 2.5 mg/d (= PN 2.0 mg/d); one mother had taken 12.5 mg/d, one mother 4.0 mg/d, and the others 2.0–2.5 mg/d PN·HCl (= PN 10.0 mg, 3.2 mg, and 1.6–2.0 mg, respectively). Five days after delivery all the mothers started a daily PN·HCl supplement of 1.0 mg (= PN 0.8 mg/d). The infants received vitamin A and D supplementation, but no vitamin B_6 supplement.

Measurements. Birth length and weight were measured by the nurses of the maternity unit. All the infants were examined and measured at 1, 2, 3, 4, 5, 6, 7.5, 9, and 12 mo by one of the authors (L.S.). Naked weight was measured to the nearest 5 g on an infant scale. Length to the nearest millimeter with the child lying on a board with fixed headpiece and a movable footpiece.

The most recent Finnish standards were used for determining length-for-age in SDS and weight-for-age in percent of median weight for age and sex. The growth velocities for 2–3-mo age intervals were calculated by dividing the increments (mm or g) by the actual time interval (d). The midparent height (SDS) was calculated.

Blood samples. Venous blood samples were taken at 2, 4, 6, 9, and 12 mo of age. For the determinations of vitamin B_6 parameters, samples of 4 mL were collected into tubes lined with sodium heparin (Becton Dickinson Vacutainer, Plymouth, UK) and centrifuged immediately at 770 × *g* for 5 min at room temperature; the cells were transferred to a sterile brown glass septum ampule containing 0.75 mL of amino acid-citric acid-dextrose solution as stabilizer. The samples, kept at 4–6°C, were transported daily by air to the Department of Clinical Nutrition, Roche Holding Ltd., Basle, Switzerland, for determinations of EPLP and the EAST stimulation test.

Protein status. Total serum protein was determined by the biuret reaction, and prealbumin and transferrin by immunoturbidimetry.

Assay of EPLP. The EPLP assay was a modified version (20) of the method of Reinken (21), based on decarboxylation of ^{14}C-L-tyrosine by reconstituted tyrosine decarboxylase after trichloroacetic acid precipitation of protein from the erythrocyte suspension. The results are expressed as nmol/L of pure erythrocytes at a calculated hematocrit of 100%. In vitamin B_6 deficiency EPLP, the active metabolite of vitamin B_6, is decreased.

EAST stimulation test. Transamination of aspartic acid to 2-oxoglutarate was monitored with ($EAST_+$) and without ($EAST_o$) excess PLP, according to a modification (20) of the method of Stanulovic *et al.* (22). Results are given as μkat/L erythrocytes, where μkat represents the amount of substrate (μmol) transformed per second. The activation coefficient αEAST was calculated. In vitamin B_6 deficiency, $EAST_o$, in which PLP acts as a coenzyme, is decreased, and αEAST, which reflects desaturation of EAST with its coenzyme, is increased.

Reference values. As reference population we used infants exclusively breast-fed up to 6 mo of age by mothers with an adequate vitamin B_6 status, given solids in addition from 6 mo of age, and weaned to cow milk via formula after 9 mo of age (23). We used the 10th percentile of the EPLP and $EAST_o$ values as cutoff points for low values, and the 90th percentile of the αEAST values as cutoff point for high values (23). Coincidence of at least two values beyond the cutoffs (Table 1) of the three was taken to indicate low vitamin B_6 status (23). As a result, we detected of the reference infants those with their vitamin B_6 status in the lowest 5–9% at each age (2, 4, 6, 9, and 12 mo) (23).

Infants with low vitamin B6 status in the present study. A low vitamin B_6 status was observed between 4 and 6 mo of age in seven ($lowB_6$; in the lowest decile for vitamin B_6 status, determined with EPLP and EAST stimulation test, in breast-fed reference infants) of the 44 infants; in three of them (7%) at 4 mo and in four (9%) at 6 mo of exclusive breast feeding (Table 1). Vitamin B_6 status of these infants was adequate at 9 and 12 mo. The group characteristics for the $lowB_6$ infants and for those who had adequate vitamin B_6 status ($adeB_6$; above the lowest decile for vitamin B_6 status, determined with EPLP and EAST stimulation test, in breast-fed reference infants) are given in Table 2. Mean length and mean length-for-age at each age were similar for the $lowB_6$ and $adeB_6$ infants.

Statistical analysis. The data were analyzed with Statview 512+ from MacIntosh (Brainpower, Calabasas, CA). We used analysis of variance for repeated measurements, the nonparametric Friedman two-way analysis of variance for repeated measurements for longitudinal evaluation of growth parameters, and *t* test and the Mann-Whitney *U* test to identify group differences in growth. Pearson correlation coefficients were calculated from regression analyses of these growth and vitamin B_6 status parameters to analyze their relations. Multiple-linear-regression analyses were performed to examine relationship between vitamin B_6 status (included as a category factor = low or adequate during exclusive breast feeding) and sub-

Table 1. *EPLP, $EAST_o$, and αEAST and longitudinal growth in lowB_6 infants during exclusive breast feeding*

$LowB_6$ infants	Sex		Vitamin B_6 status 2 mo	4 mo	6 mo	9 mo	12 mo	Length velocity (mm/d) 2–4 mo	4–6 mo	6–9 mo	9–12 mo	Length-for-age (SDS) 0 mo	1 mo	2 mo	3 mo	4 mo	5 mo	6 mo	7.5 mo	9 mo	12 mo	Midparent height (SDS)
1	M	EPLP (nmol/L)	40.5*	60.7	36.4*	62.7	65.8															
		$EAST_o$ (μkat/L)	10.2	17.1	9.6*	14.1	15.3	1.08	0.42	0.17	0.73	+0.2	+0.6	+1.0	+1.1	+1.2	+0.5	+0.2	−0.7	−1.0	+0.4	+0.5
		αEAST	1.75	1.47	1.73*	1.56	1.57															
2	M	EPLP (nmol/L)	38.4*	64.8	41.5*		84.0															
		$EAST_o$ (μkat/L)	12.0	16.0	12.4*		16.4	0.81	0.63	0.52	0.43	+0.2	+0.1	+0.5	−0.1	−0.2	−0.2	−0.5	−0.4	−0.4	−0.2	+0.2
		αEAST	1.73	1.48	1.65		1.59															
3	F	EPLP (nmol/L)	95.1	78.9	42.5*	64.8	58.7															
		$EAST_o$ (μkat/L)	16.2	12.8*	12.9*	15.1	15.9	0.66	0.65	0.38	0.48	−0.9	−0.6	−0.5	−0.8	−1.6	−1.0	−1.5	−2.0	−2.0	−1.4	−0.2
		αEAST	1.69	1.41	1.61	1.56	1.67															
4	M	EPLP (nmol/L)	52.6	72.8	43.5*	64.8	62.7															
		$EAST_o$ (μkat/L)	11.8	16.8	11.8*	16.2	16.0	0.78	0.57	0.28	0.39	−0.1	+0.1	+1.0	−0.1	0.0	−0.4	−0.5	−0.9	−1.3	−1.0	+1.1
		αEAST	1.53	1.50	1.73*	1.55	1.52															
5	M	EPLP (nmol/L)	101.2	71.8	67.8	74.9	58.0															
		$EAST_o$ (μkat/L)	14.6	15.8*	15.0	14.7	12.1*	0.82	0.78	0.16	0.51	+1.2	+0.6	+0.5	+0.4	−0.2	−0.3	−0.3	−1.1	−1.5	−1.1	+0.3
		αEAST	1.43	1.68*	1.63	1.47	1.72															
6	F	EPLP (nmol/L)		59.7*	55.6	62.7	39.5*															
		$EAST_o$ (μkat/L)		13.8*	13.5	15.8	12.9	1.02	0.67	0.19	0.45	+0.4	+1.3	+1.6	+1.2	+1.4	+0.9	+1.1	+0.4	0.0	0.0	+0.2
		αEAST		1.58*	1.66	1.60	1.63															
7	M	EPLP (nmol/L)		58.7*	54.6	57.7	40.5*															
		$EAST_o$ (μkat/L)		15.8*	13.2	14.8	12.9	0.71	0.61	0.39	0.47	+0.2	+0.6	+0.8	−0.1	−0.5	−0.6	−0.9	−1.1	−1.0	−0.7	+0.2
		αEAST		1.59	1.67	1.64	1.82															
Cutoffs used		EPLP (nmol/L)	41.5	59.7	51.6	47.8	51.6															
		$EAST_o$ (μkat/L)	10.1	16.0	13.0	14.1	12.7															
		αEAST	1.76	1.54	1.68	1.64	1.84															

* EPLP or $EAST_o$ < the 10th percentile or αEAST > the 90th percentile of the values for respective parameters in the breast-fed reference infants at the same age. Coincidence of such values for at least two of the three parameters detected the reference infants with their vitamin B_6 status in the lowest 5–9%.

Table 2. *Group characteristics of infants with adequate ($adeB_6$) and low ($lowB_6$) vitamin B_6 status during breast feeding*

	$AdeB_6$ (n = 37)	$LowB_6$ (n = 7)
Maternal age (y)	30.1 ± 1.2	31.7 ± 1.8
Parity	1.3 ± 0.1 (1–3)	2.0 ± 0.3 (1–3)**
Sex (females:males)	22:15	2:5
Gestational vitamin B_6 supplement	24/37 (=65%)	6/7 (=85%)
Birth weight (g)	3438 ± 39	3741 ± 78*
Relative birth weight (% of median)	−1.5 ± 1.1	6.3 ± 2.3*
Birth length (cm)	50.1 ± 0.2	50.9 ± 0.5
Relative birth length (SD)	−0.14 ± 0.11	0.17 ± 0.24
Midparent height (SD)	0.14 ± 0.07	0.32 ± 0.16
Age at introduction of formula (d)	272 ± 5	282 ± 12
Age at completion of weaning (d)	353 ± 13	359 ± 21
Low vitamin B_6 status at 4 mo (n)	0	3
Low vitamin B_6 status at 6 mo (n)	0	4
Low vitamin B_6 status at 9–12 mo (n)	0	0

Difference between the groups statistically significant; t test, * $p \leq 0.05$, ** $p \leq 0.01$.

sequent longitudinal growth. Potentially confounding factors (sex, size at birth, previous gain in weight and length, and midparent height) were included in the analyses. In the forward stepwise multiple-regression analyses, inclusion/exclusion criteria were partial $F = 4$ and $p \leq 0.05$. The χ^2 test was used to evaluate the differences in gestational supplementation and sex distribution between the groups. The level of significance was set at $p \leq 0.05$ for all statistical analyses.

RESULTS

Growth patterns. Male and female subjects differed only in mean birth length (50.8 *versus* 49.8 cm, $p \leq 0.01$), in mean weight velocity from birth to 2 mo (33.0 *g*/d *versus* 27.7 *g*/d; $p \leq 0.007$), and in mean length velocity from birth to 6 mo of age ($p \leq 0.005$), but the difference was not significant when length velocity was compared at 2–3-mo age intervals. Thus, the data for boys and girls were generally combined for analyses. Mean weight- and length-for-age increased from birth to 2 mo, and then decreased gradually until 9 mo (Figs. 1 and 2). Absolute and relative birth weights correlated inversely with weight velocity and with change in weight-for-age, but this correlation weakened with age. Concomitant weight velocity and changes in weight-for-age correlated with length velocity and changes in length-for-age throughout infancy.

Associations between single vitamin B6 parameters and growth. There was an inverse correlation between birth weight and vitamin B_6 status at 2 mo (EPLP $r = -0.35$, $p \leq 0.04$; $EAST_o$ $r = -0.40$, $p \leq 0.02$; αEAST $r = 0.46$, $p \leq 0.01$). The higher the preceding weight velocity and the increase in weight-for-age, the higher the vitamin B_6 status at 2–6 mo ($r = 0.30$–0.52, $p \leq 0.001$–0.05).

At 4 mo, EPLP correlated positively with length velocity from birth to 6 mo ($r = 0.43$, $p \leq 0.006$). At 6 mo, EPLP correlated positively with length velocity and with change in length-for-age from birth to 9 mo ($r = 0.31$ to 0.34, $p \leq 0.03$–0.04), and $EAST_o$ with length velocity and with change in length-for-age from 6 to 9 mo ($r = 0.31$–0.32, $p \leq 0.05$).

Gain in weight and length in infants with adequate and low vitamin B6 status. From 1 to 12 mo, mean weight and mean weight-for-age did not differ in the $lowB_6$ and the $adeB_6$ infants, even though the mean absolute and relative birth weights were higher in the $lowB_6$ than in the $adeB_6$ infants (mean ± SEM 3741 ± 78 g *versus* 3438 ± 39 g, $p \leq 0.003$, and 6.3 ± 2.3% *versus* −1.5 ± 1.1%, $p \leq 0.008$, respectively, Table 2). By 9 mo of age, mean weight-for-age had fallen more in the $lowB_6$ than in the $adeB_6$ infants ($p \leq 0.02$; Fig. 1). The difference between their weight velocities when determined for 2–3-mo age intervals were not statistically significant (Table 3).

From birth to 6 mo, mean length increments (mm), mean length velocity (Table 3), and changes in length-for-age in the $lowB_6$ infants did not differ from those in the $adeB_6$ infants.

Between 6 and 9 mo the $lowB_6$ infants grew more slowly than the $adeB_6$ infants. Mean (±SEM) length increment was smaller (2.7 ± 0.4 *versus* 3.7 ± 0.2 mm, $p \leq 0.02$), and length velocity was lower (0.30 ± 0.05 *versus* 0.40 ± 0.02 mm/d, $p \leq 0.02$; Table 3) in the $lowB_6$ than in the $adeB_6$ infants. Furthermore, mean (±SEM) length-for-age fell more sharply

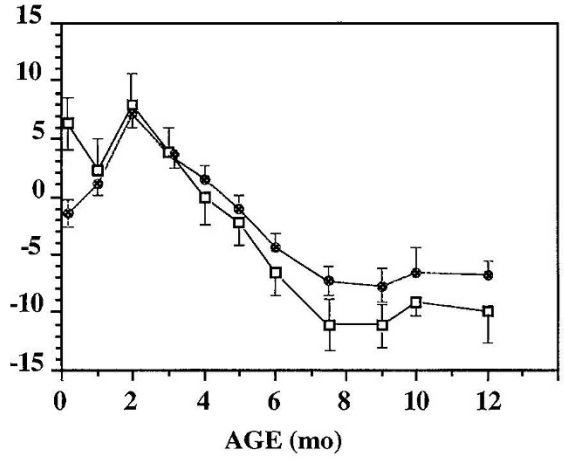

Figure 1. Mean (± SEM) weight-for-age (% of median) during the first year of life in infants with adequate (●) and with low (□) vitamin B_6 status during breast feeding.

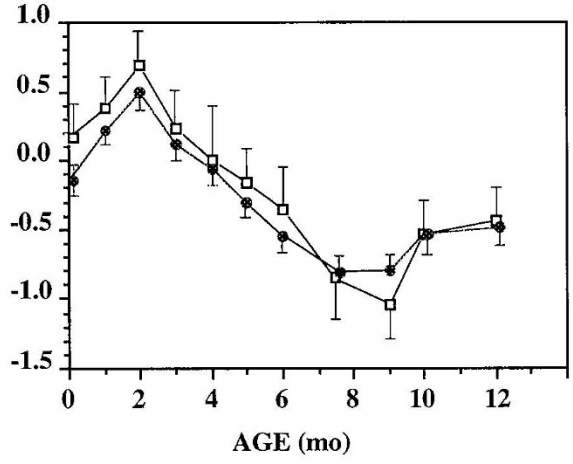

Figure 2. Mean (± SEM) length-for-age (SDS) during the first year of life in infants with adequate (●) and with low (□) vitamin B_6 status during breast feeding.

Table 3. *Mean (±SEM) length and weight velocity determined for 2–3-mo age intervals in infants with adequate ($adeB_6$) and with low ($lowB_6$) vitamin B_6 status*

Growth velocity/age interval	$AdeB_6$ infants	$LowB_6$ infants
Length velocity (mm/d)		
0–2 mo	1.32 ± 0.03	1.32 ± 0.07
2–4 mo	0.86 ± 0.02	0.84 ± 0.06
4–6 mo	0.59 ± 0.02	0.62 ± 0.04
6–9 mo	0.40 ± 0.02	0.30 ± 0.05*
9–12 mo	0.45 ± 0.02	0.49 ± 0.04
Weight velocity (g/d)		
0–2 mo	30.6 ± 1.1	27.8 ± 2.6
2–4 mo	22.3 ± 0.8	20.6 ± 1.9
4–6 mo	15.2 ± 0.8	14.0 ± 2.0
6–9 mo	10.1 ± 0.6	8.9 ± 0.5
9–12 mo	9.9 ± 0.4	9.4 ± 2.2

* Difference between the groups statistically significant; *t* test, $p \leq 0.05$.

in the $lowB_6$ than in the $adeB_6$ infants (−0.69 ± 0.20 *versus* −0.25 ± 0.07 SDS, $p \leq 0.03$) between 6 and 9 mo (Fig. 2). A decrease in SDS of > 1.0 SD from birth occurred by 6 mo in 30% and by 9 mo in 71% of the $lowB_6$ infants *versus* 13 and 30%, respectively, of the $adeB_6$ infants.

In the $lowB_6$ infants, mean length velocity was higher between 9 and 12 mo than between 6 and 9 mo ($p \leq 0.05$), but in the $adeB_6$ infants it was stable between 6 and 12 mo. In all the $lowB_6$ infants length-for-age increased from 9 to 12 mo. In two of them, length velocity was 3–4-fold higher between 9 and 12 mo than between 6 and 9 mo, and length-for-age increased by 1.4 and 1.5 SD units between 9 and 12 mo of age. Individual data on longitudinal growth of the $lowB_6$ infants is shown in Table 1.

Longitudinal growth in infants with low and adequate vitamin B6 status. Length velocity and change in length-for-age between 6 and 9 mo correlated positively with preceding and concomitant weight velocity (4–9 mo), change in weight-for-age (4–9 mo; $r = 0.41–0.45$, $p \leq 0.003–0.05$), and preceding vitamin B_6 status (determined at each age concomitantly with EPLP and EAST stimulation test, and used as a category factor; adequate or low at 4–6 mo; Spearman $r = 0.28–0.31$, $p \leq 0.05–0.07$). In addition, change in length-for-age (6–9 mo) correlated inversely with absolute and relative birth weights ($r = −0.31$, $p \leq 0.05$) and with difference between length-for-age (6 mo) and midparent height ($r = −0.33$, $p \leq 0.03$).

Of the analyzed factors, length gain correlated best with weight gain, and better with change in weight-for-age than with weight velocity. Further, the change in weight-for-age during the age period 4–9 mo had a higher correlation with longitudinal growth between 6 and 9 mo than changes in weight-for-age during any other age periods. Therefore, change in weight-for-age from 4 to 9 mo (partly reflecting total energy and protein intake) was included in the multiple regression analyses for length velocity (6–9 mo) and for change in length-for-age (6–9 mo), as well as preceding vitamin B_6 status (adequate or low at 4–6 mo) as explanatory factors. As possible confounding factors, sex, relative birth weight (observed difference between the $adeB_6$ and the $lowB_6$ infants), and difference between length-for-age at 6 mo and midparent height (reflecting preceding longitudinal growth and possible tendency to grow toward midparent height) were also included in the analyses. The strongest explanatory factor for length velocity and for change in length-for-age between 6 and 9 mo was change in weight-for-age (4–9 mo). The association between vitamin B_6 status (4–6 mo) and length velocity or length-for-age (6–9 mo) could not be explained by variation in the other variables included in the analyses. In the forward stepwise multiple regression analyses, change in weight-for-age explained 16 and 18%, and its combination with preceding vitamin B_6 status 23 and 27%, of the variation in length velocity and in change in length-for-age, respectively (Tables 4 and 5).

Vitamin B6 status and longitudinal growth in pairs matched for birth weight. To exclude the possibility that our results were affected by the difference in birth weight between the $lowB_6$ and the $adeB_6$ infants, we selected for each of the seven $lowB_6$ infants pairs matched for sex and birth weight from among the $adeB_6$ infants. The $lowB_6$ infants had a lower length velocity (0.30 ± 0.05 *versus* 0.41 ± 0.16 mm/d; one-tailed *t* test, $p \leq 0.06$) and a deeper fall in length-for-age from 6 to 9 mo of age (−0.69 ± 0.20 *versus* −0.25 ± 0.16 SDS; one-tailed *t* test, $p \leq 0.06$) than their matched $adeB_6$ infant pairs.

Table 4. *Multiple linear regression analysis for different variables explaining length velocity between 6 and 9 mo of age*

Explanatory variable	Regression coefficient (± SEM)	Partial *F*	*p*
Response variable: length velocity between 6 and 9 mo (mm/d)			
Multiple linear regression analysis: $R = 0.57$, adjusted $R^2 = 0.23$, $p \leq 0.01$			
Sex	0.04 ± 0.03	1.92	0.18
Relative birth weight (% of median)	0.002 ± 0.002	0.47	0.50
Vitamin B_6 status at 4–6 mo (low/adequate)	0.11 ± 0.04	6.44	0.02*
Difference between midparental height and length-for-age at 6 mo	−0.02 ± 0.02	1.28	0.27
Change in weight-for-age from 4 to 9 mo	0.009 ± 0.003	6.46	0.02*
Forward stepwise multiple regression analysis (inclusion criteria: $F = 4.0$, $p \leq 0.05$)			
Step 1 ($R = 0.42$, adjusted $R^2 = 0.16$)			
Change in weight-for-age from 4 to 9 mo	0.010 ± 0.003	8.91	0.003*
Step 2 ($R = 0.52$, adjusted $R^2 = 0.23$, $p \leq 0.002$)			
Change in weight-for-age from 4 to 9 mo	0.009 ± 0.003	7.62	0.01**
Vitamin B_6 status at 4–6 mo (low/adequate)	0.09 ± 0.04	4.84	0.03*

Significant explanatory factor; * $p \leq 0.05$, ** $p \leq 0.01$.

Table 5. *Multiple linear regression analysis of different variables explaining change in length-for-age between 6 and 9 mo of age*

Explanatory variable	Regression coefficient (± SEM)	Partial F	p
Response variable: change in length-for-age between 6 and 9 mo (SD)			
Multiple linear regression analysis: $R = 0.59$, adjusted $R^2 = 0.27$, $p \leq 0.005$			
Sex	0.16 ± 0.14	1.46	0.23
Relative birth weight (% of median)	−0.001 ± 0.01	0.02	0.89
Vitamin B_6 status at 4–6 mo (low/adequate)	0.41 ± 0.19	4.42	0.04*
Difference between midparental height and length-for-age at 6 mo (SD)	−0.15 ± 0.09	2.63	0.11
Change in weight-for-age from 4 to 9 mo	0.04 ± 0.02	5.86	0.02*
Forward stepwise multiple regression analysis (inclusion criteria: partial $F = 4.0$, $p \leq 0.05$)			
Step 1 ($R = 0.45$, adjusted $R^2 = 0.18$)			
Change in weight-for-age from 4 to 9 mo	0.047 ± 0.015	10.32	0.003**
Step 2 ($R = 0.53$, adjusted $R^2 = 0.24$, $p \leq 0.002$)			
Change in weight-for-age from 4 to 9 mo	0.043 ± 0.014	8.96	0.005**
Vitamin B_6 status at 4–6 mo (low/adequate)	0.36 ± 0.18	4.14	0.05*

Significant explanatory factor; * $p \leq 0.05$, ** $p \leq 0.01$.

Protein status in infants with low and adequate vitamin B_6 status. The lowB_6 and the adeB_6 infants did not differ in plasma protein, prealbumin, or transferrin concentrations at 4 mo (60 ± 2 *versus* 60 ± 1 g/L, 182 ± 19 *versus*, 175 ± 6 mg/L, and 2.5 ± 0.2 *versus* 2.5 ± 0.1 mg/L, respectively), at 6 mo (63 ± 1 *versus* 62 ± 1 g/L, 173 ± 20 *versus* 167 ± 4 mg/L, and 2.6 ± 0.2 *versus* 2.6 ± 0.1 mg/L, respectively), or at 9 mo (63 ± 1 *versus* 62 ± 1 g/L, 201 ± 2 *versus* 197 ± 10 mg/L, and 3.0 ± 0.2 *versus* 2.8 ± 0.1 mg/L, respectively).

DISCUSSION

General growth pattern. For this study, we selected a group of infants standardized for type of feed to minimize the intragroup variation in the intake of nutrients. Their feeding pattern was in accord with the current recommendations by WHO and, as a group, these infants grew as expected for breast-fed infants (3, 4). During exclusive breast feeding their weight-for-age percentiles fell, and the fall continued well after the introduction of solid foods. Maximal length-for-age was attained by 2 mo, as in the DARLING study infants, and the SDS then fell for longer and deeper, possibly because solid foods were introduced later than in the DARLING study. Weight velocity and length velocity calculated for 2–3-mo age intervals were similar to those observed in breast-fed infants (5, 9).

Slower growth rate in infants with low vitamin B6 status. A new observation is that, in breast-fed infants, a low vitamin B_6 status is associated with subsequently slowed length velocity and a deeper fall in length-for-age. This is in accord with the earlier described correlation between vitamin B_6 intake and length gain in breast-fed neonates (18).

Weight gain has been observed to correlate with vitamin B_6 status both in animal experiments and in human neonates (14, 18). In our study, the breast-fed infants with a low vitamin B_6 status had a poorer weight gain than those with an adequate status. This difference became evident even during the first 4 mo and in all probability was mostly due to the difference in mean birth weight between the groups. On the other hand, there was a constant positive correlation between parameters reflecting long-term vitamin B_6 status and preceding weight gain. The association reflects most probably the amount and quality of breast milk (24).

Because there are no studies about metabolic or hormonal effects of vitamin B_6 deficiency in human infants, we can only speculate on the mechanisms underlying the association between vitamin B_6 and infant growth. Vitamin B_6 acts as a coenzyme in protein, carbohydrate, and fat metabolism. The higher the protein intake, the higher the requirement for vitamin B_6 or the earlier the signs of the vitamin deficiency. Thus, low vitamin B_6 status could lead in ineffective utilization of dietary proteins for growth. In animal studies, vitamin B_6 deficiency has also been shown to produce alterations in action of hormones, including insulin, and thyroid hormone (25).

The possible effects of low vitamin B_6 status on utilization of dietary proteins and on the hormonal balance could partly explain poorer growth of our lowB_6 infants, and be involved in causing the difference in growth pattern between breast- and formula-fed infants and, especially, the growth faltering of some breast-fed infants (26).

Reversible threshold phenomenon not resulting in stunting. Vitamin B_6 does not seem to be a growth factor. The association between longitudinal growth and individual vitamin B_6 parameters appeared only during the period in which low vitamin B_6 status was observed, and reduced length velocity was later observed in those infants in whom at least two of the three vitamin B_6 parameters were beyond the used cutoffs. This suggests that longitudinal growth is affected by vitamin B_6 status only beneath a certain threshold level and after a delay. In animal and human studies, vitamin B_6 deficiency has been shown to result in decreased weight gain only after a delay (14, 17) which is the shorter, the higher the dietary protein intake (14). Our infants with a low vitamin B_6 status between 4 and 6 mo also had a low protein intake due to exclusive breast feeding. When additional solid foods were introduced and protein intake thus gradually increased, their longitudinal growth slowed down and length-for-age decreased further.

The slowing of growth was reversible and did not result in shorter stature at the end of the first year. After 6 mo of age, the infants received increasing amounts of solids and were weaned via formula to cow milk, presumably resulting in a gradually increasing intake of total energy, protein (8), and vitamin B_6.

Vitamin B_6 status improved by 9 mo, and thereafter length velocity recovered and length-for-age returned to earlier levels.

Confounding factors influencing growth. A fact that complicates evaluation of our findings is the higher mean birth weight of the lowB_6 infants compared with the adeB_6 infants. Extenuating facts are that the negative correlation between birth weight and growth was evident only during the first 2 mo and that from 2 mo of age the groups did not differ in absolute or relative weight. Nor did birth weight correlate with length velocity at any age. Furthermore, in multiple regression analysis, the negative correlation between birth weight and changes in length-for-age between 6 and 9 mo was explained by variation in the other explanatory variables. As expected, the preceding and concomitant change in weight-for-age strongly explained both length velocity and change in length-for-age. In addition, preceding vitamin B_6 status remained a significant explanatory factor for longitudinal growth from 6 to 9 mo of age even though factors earlier reported to influence growth were taken into account, including sex, size at birth, preceding and concomitant weight gain (partly reflecting total energy and protein intake), and inherited growth pattern. Furthermore, similar plasma prealbumin and transferrin levels of the groups strengthen the view that the growth difference was not due to significantly different protein or energy intake.

To conclude: in healthy breast-fed infants, a low vitamin B_6 status seems to be associated with reversible reduction of length velocity accompanied by decrease in length-for-age. This association and the underlying mechanisms need further investigation.

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