Vitamin B₆ Vitamer Concentrations in Cerebrospinal Fluid Differ Between Preterm and Term Newborn Infants

WHAT’S KNOWN ON THIS SUBJECT: There is no literature on the concentrations of vitamin B₆ vitamers in cerebrospinal fluid of preterm and term newborn infants. This knowledge, however, is highly important, because vitamin B₆ plays a pivotal role in brain development and functioning.

WHAT THIS STUDY ADDS: In cerebrospinal fluid of newborn infants, B₆ vitamer concentrations are strongly dependent on postmenstrual age, indicating that vitamin B₆ homeostasis in brain differs between preterm and term newborns. This has implications for the evaluation of epilepsy and vitamin B₆ deficiency.

abstract

BACKGROUND AND OBJECTIVE: Vitamin B₆ plays a pivotal role in brain development and functioning. Differences in vitamin B₆ homeostasis between preterm and term newborn infants have been reported. The authors sought to investigate whether B₆ vitamers in cerebrospinal fluid (CSF) of preterm and term newborn infants are different.

METHODS: B₆ vitamer concentrations were determined in 69 CSF samples of 36 newborn infants (26 born preterm and 10 born term) by ultra performance liquid chromatography-tandem mass spectrometry. CSF samples, taken from a subcutaneous intraventricular reservoir, were bedside frozen and protected from light.

RESULTS: Concentrations of pyridoxal (PL), pyridoxal phosphate (PLP), pyridoxic acid (PA), and pyridoxamine (PM) in preterm newborns (postmenstrual age 30–37 weeks) were at least twice as high as in older newborns (postmenstrual age >42 weeks). Pyridoxine concentrations were below limits of quantification in all newborns. In CSF of 2 very preterm newborns (postmenstrual age <30 weeks), significant amounts of pyridoxine were present besides high concentrations of PL, PA, and PM, whereas PLP concentrations were relatively low. B₆ vitamers in CSF were positively correlated, especially PA, PLP, and PL.

CONCLUSIONS: In CSF of newborn infants, PL, PLP, PA, and PM are present, and concentrations are strongly dependent on postmenstrual age. Our results indicate that vitamin B₆ homeostasis in brain differs between preterm and term newborns. These results should be taken into account for diagnosis and treatment of epilepsy and vitamin B₆ deficiency in newborn infants. Pediatrics 2012;130:e191–e198

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KEY WORDS vitamin B₆, cerebrospinal fluid, newborn infants

ABBREVIATIONS CSF—cerebrospinal fluid GA—gestational age PL—pyridoxal PLP—pyridoxal phosphate PA—pyridoxic acid PM—pyridoxamine PN—pyridoxine PMP—pyridoxamine phosphate

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Vitamin B₆ is a water-soluble and, for humans, essential nutrient. It comprises different vitamers: the alcohol pyridoxine (PN), the aldehyde pyridoxal (PL), the amine pyridoxamine (PM), and their phosphate-esterified forms. Pyridoxic acid (PA) is the major degradation product of vitamin B₆ that is excreted in urine. Pyridoxal phosphate (PLP), is well known as a cofactor to a large number of essential enzymatic reactions in the central nervous system that catalyze amino acid and neurotransmitter metabolism.

Before transport across membranes of brain cells and choroid plexus, phosphorylated B₆ vitamers must be hydrolyzed by a membrane-bound alkaline phosphatase. Intracellular (re-)phosphorylation by pyridoxal kinase is followed by a pyridox(am)ine phosphate oxidase–mediated conversion of pyridoxine phosphate (PNP) and pyridoxamine phosphate (PMP) into PLP. Brain cells only release the unphosphorylated B₆ vitamers after hydrolysis by a specific phosphatase, whereas choroid plexus releases phosphorylated as well as unphosphorylated B₆ vitamers into cerebrospinal fluid (CSF).

PLP is important for the biosynthesis of dopamine, serotonin, glutamate, γ-aminobutyrate, histamine, and α-serine, which suggests a crucial role in brain development and functioning. Several studies performed in rats indeed have shown lower brain weights, impaired neuromotor development, and seizures after prenatal induction and postnatal maintenance of dietary vitamin B₆ deficiency. In addition, adverse effects on neurogenesis, neuronal longevity, neuronal differentiation, and synaptogenesis have been described, and reduced myelination, as well. In humans, genetic vitamin B₆ deficiency results in seizures, which can be accompanied by variable degrees of structural brain abnormalities and psychomotor retardation. Several autosomal recessive disorders are known to be causative, such as antiquitin deficiency, a disorder of cerebral lysine degradation caused by mutations in the ALDH7A1 gene, and pyridox(am)ine phosphate oxidase deficiency, caused by mutations in the PNPO gene. Whereas seizures due to antiquitin deficiency are responsive to PN, pyridoxal (phosphate) (PL(P)) must be administered in case of PNPO deficiency. Other genetic causes of vitamin B₆ deficiency are hyperprolinemia type II and hypophosphatasia (tissue nonspecific alkaline phosphatase deficiency).

Because vitamin B₆ is crucial for brain development and functioning, knowledge on vitamin B₆ homeostasis in healthy newborn infants is clinically highly important. In this context, the studies of Raiten et al are interesting, because they reported low concentrations of PLP in serum of preterm newborns <30 weeks of gestational age (GA), whereas serum PLP concentrations were initially low, but increased significantly after pyridoxine supplementation. In preterm newborns <28 weeks of GA, supplementation of pyridoxal did not induce a serum PLP response either. In these studies, no information was obtained on brain homeostasis of vitamin B₆.

The findings of Raiten et al triggered us to study vitamin B₆ homeostasis in newborn infants. We used CSF samples, because they provide the only accessible reflection of brain homeostasis in humans. We compared B₆ vitamers in CSF of preterm and term newborn infants.

**METHODS**

We obtained 69 remnant CSF samples of 36 newborn infants (18 female, 18 male). Twenty-six infants were born preterm (<37 weeks of gestation) and 10 were born term (≥37 weeks of gestation). The youngest preterm infant was born at 26 weeks of gestation, whereas the oldest term infant was born at 41 weeks. Postnatal age at CSF withdrawal...
ranged from 2 to 153 days, and postmenstrual age (postnatal age corrected for duration of pregnancy) ranged from 28–42 weeks to 53–45 weeks.

Newborn infants were admitted to the NICU of the Wilhelmina Children’s Hospital, University Medical Center Utrecht, Netherlands. Indication for CSF withdrawal was a posthemorrhagic ventricular dilatation (n = 33) or congenital hydrocephaly (n = 3) with the necessity to remove CSF at regular intervals from a subcutaneous intraventricular reservoir to avoid or alleviate raised intracranial pressure. CSF samples were collected between 2005 and 2010. Immediately after withdrawal, the samples were centrifuged (1200 × g), protected from light, and stored at −80°C. Parental informed consent was obtained to use remnants of clinically obtained CSF samples. Approval by the Medical Ethics Committee of the University Medical Center Utrecht was obtained. B6 vitamer (PL(P), PM(P), PN, and PA) concentrations were determined by using a sensitive and accurate ultra performance liquid chromatography-tandem mass spectrometry method with stable isotope-labeled internal standards. Twenty-nine newborn infants were breastfed (with or without addition of breast milk fortifier [50 µg pyridoxine/100 mL] and/or received (preterm) infant formula containing 40 to 120 µg pyridoxine/100 mL). Seven newborns also observed between term newborns and preterm, and older newborns (postmenstrual age, ≥42 weeks) (Table 2, Fig 4). A strong correlation was observed between the concentration of the active cofactor PLP, its direct precursor PL, and the concentration of the degradation product PA (Spearman ρ 0.631 for PLP and PL, 0.849 for PA and PL, respectively; P < .0005). The ratio between PA and PL was higher in preterm (postmenstrual age, 30–37 weeks) and term (age, 37–42 weeks) newborns compared with older newborns (age, ≥42 weeks) (P < .0005 and P < .009, respectively; Mann-Whitney U tests with Bonferroni correction [cutoff significance level P = .017]). The ratio between PLP and PL did not depend on postmenstrual age (Table 1). In both very preterm newborns A and B, correlations of PA, PLP, and PL and ratios between these B6 vitamers were different in comparison with newborn infants with a postmenstrual age >30 weeks (Table 1, Fig 4).

B6 vitamer concentrations were determined in 69 CSF samples of 36 newborn infants. Because the number of CSF samples of each newborn ranged from 1 to 5 at different postmenstrual ages, it is likely that subsequent CSF samples of an individual newborn belonged to the same subgroup. Exclusion of all subsequent CSF samples after initial CSF withdrawal from each newborn for each subgroup, however, did not significantly change B6 vitamer concentration differences between subgroups (n = 45). In fact, those individual newborns from whom multiple CSF samples were collected, all showed a decrease of B6 vitamer concentrations with postmenstrual age (data not shown).

Remarkably, in CSF of 2 newborns on parenteral nutrition (n = 2), PN was
present in quantifiable amounts (0.07 and 0.12 nM), whereas it was below limits of quantification (0.03 nM) in all other newborns with a postmenstrual age ≥30 weeks (n = 65).

**DISCUSSION**

In CSF of newborn infants, we found B6 vitamer concentrations of PL > PLP > PA > PM, showing that vitamin B6 in CSF is mainly present as its active cofactor and direct precursor (PLP and PL, respectively). This is in agreement with our recently reported B6 vitamer concentrations in CSF of children aged 8 months to 16 years (n = 20).\(^{20}\) Interestingly, in neocortex of 30-day-old rat pups, B6 vitamer concentrations have been reported to be PMP > PM > PLP > PL, with PMP being ~64% of total vitamin B6. PN was not detectable, and PNP and PA levels were not measured.\(^{10}\)

Surprisingly, concentrations of not only PL (P), but also PA and PM were much higher in newborn infants than in our group of older children.\(^{20}\) PLP concentrations were also higher than those reported in other studies,\(^{22,23}\) in which CSF of children in different age categories was analyzed. In our subgroup of newborn infants aged ≥42 weeks, B6 vitamer concentrations were still high, but they showed a strong decrease toward our recently reported concentrations in older children\(^{20}\) (Table 3).

In this study, CSF was withdrawn from a subcutaneous intraventricular reservoir, whereas, in our previous study, we used CSF obtained by lumbar puncture.
Because concentrations are not affected by a rostrocaudal gradient, B6 vitamers can be measured in a random CSF sample. Intraventricular CSF withdrawal can therefore not explain the observed differences in B6 vitamer concentrations between newborn infants and older children. This conclusion is further strengthened by the observation that concentrations in newborns aged 42 weeks (CSF withdrawn from subcutaneous intraventricular reservoir) strongly decrease to reach concentrations in older children (CSF obtained by lumbar puncture).

We investigated whether the amounts of cells and protein in CSF influenced B6 vitamer concentrations. Erythrocyte, leukocyte, and protein concentrations in 67 of 69 CSF samples were increased (10^6/mL, 10^5/mL, and 0.40 mg/mL, respectively). However, no correlation of erythrocyte, leukocyte, and protein content of CSF with B6 vitamer concentrations was observed (Spearman r, 0.500 and/or P > .05), making a confounding effect of cell and protein contamination on B6 vitamer concentrations in CSF of newborn infants unlikely.

Nutrition is another possible confounding factor. Breast milk is known to contain primarily pyridoxal, whereas breast milk fortifier and (preterm) infant formula contain pyridoxine (40–120 μg/100 mL). Parenteral nutrition also provides the newborn infant with pyridoxine (490 μg/kg body weight per day). Although the type of nutrition differed between individual newborns, B6 vitamer and especially PLP concentrations did not depend on the type of nutrition (data not shown).

The 2 very preterm newborns (postmenstrual age, <30 weeks) both showed a different B6 vitamer profile of PL > PA >
PLP > PM > PN in CSF. Concentrations of PL, PA, and PM were higher than in newborns with a postmenstrual age >30 weeks, PN was present in quantifiable amounts, and PLP was relatively low. Both these newborns received parenteral nutrition (490 μg pyridoxine/kg body weight per day). Only in case of newborn B there was prenatal maternal supplementation with pyridoxine (25–50 mg/day; indication, nausea of pregnancy). CSF samples were taken 8 (newborn A) and 19 (newborn B) days postnatally. The similarity between the B₆ vitamer profiles in newborns A and B and the long period between birth and CSF withdrawal led us to the hypothesis that maternal supplementation is not likely to be the cause of the observed differences in B₆ vitamer profiles in these very preterm newborns.

The observed higher concentrations of only the unphosphorylated B₆ vitamers, including PN, in CSF of these very preterm newborns suggested a possible association with the developmental stage, since the postmenstrual age at birth was <30 weeks and the gestational age was only 21 weeks. This finding is consistent with the reported increase in PN concentrations in CSF of premature newborns compared to term newborns, which is postulated to be due to the delayed development of the central nervous system.
preterm newborns may point to an immaturity of the enzymatic system involved in vitamin B₆ homeostasis at a lower postmenstrual age. This is strengthened by the fact that B₆ vitamer concentrations in newborns (postmenstrual age, 30–37 weeks) were consistent with values found in the subgroup of preterm newborns (postmenstrual age, 30–37 weeks).

This observation is also in line with the study of Raiten et al.,¹⁹ who found continuously low serum PLP concentrations in preterm newborn infants <30 weeks of GA, and PLP concentrations remained low even after supplementation with pyridoxal as well as pyridoxine. In these infants, however, erythrocyte PLP concentrations increased with pyridoxine supplementation.¹⁹ The authors suggest that increased tissue demands or metabolite trapping of PLP and/or precursors by peripheral tissues and/or erythrocytes could underlie these observations.

Because conversion of PL into PLP is independent of the pyridox(am)ine phosphate oxidase enzyme (Fig 1), possible explanations for the failure of pyridoxal to induce a serum PLP response could be a decreased activity of pyridoxal kinase or an increased activity of the (either membrane-bound alkaline or intracellular specific) phosphatase, responsible, respectively, for phosphorylation of PL and hydrolysis of PLP; the latter of which is necessary for transport of vitamin B₆ into and out of brain cells. Both these possibilities, which are in great part also discussed by Raiten et al.,¹⁹ would fit our observation of higher concentrations of the unphosphorylated B₆ vitamers, including PL, in the presence of relatively low PLP in CSF of very preterm newborns. However, the literature is inconsistent regarding the time span of development of the enzymatic system involved in vitamin B₆ homeostasis throughout gestation.

Investigation of brain homeostasis in newborn infants is extremely challenging, but clinically highly important. We were able to collect a unique set of CSF samples from preterm and term newborns who had a subcutaneous intraventricular reservoir to lower intracranial pressure by CSF withdrawal. We used these samples to study vitamin B₆ homeostasis in newborn infants, because differences between preterm and term newborns have been suggested. Interpretation of our findings requires some caution because CSF could only be obtained from newborns with a posthemorrhagic ventricular dilatation or congenital hydrocephaly. However, this is considered for the whole group studied, and our approach provides the best possible reflection of brain homeostasis in healthy newborn infants.

CONCLUSIONS

Our results indicate that vitamin B₆ homeostasis in brain differs substantially between preterm and term newborn infants. Vitamin B₆ vitamer reference values for older children are inappropriate for application in newborns, and age-specific B₆ vitamer reference values, taking postmenstrual age into account, are indispensable for diagnosis and treatment of epilepsy and vitamin B₆ deficiency in newborn infants.

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