Vitamin B-6 vitamers in human plasma and cerebrospinal fluid

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ABSTRACT

Background: Vitamin B-6 comprises a group of 6 interrelated vitamers and is essential for numerous physiologic processes, including brain functioning. Genetic disorders disrupting vitamin B-6 metabolism have severe clinical consequences.

Objective: To adequately diagnose known and novel disorders in vitamin B-6 metabolism, a reference set is required containing information on all vitamin B-6 vitamers in plasma and cerebrospinal fluid (CSF).

Design: Concentrations of vitamin B-6 vitamers in the plasma and CSF of 533 adult subjects were measured by ultra high-performance liquid chromatography–tandem mass spectrometry.

Results: The relative vitamin B-6 vitamer composition of plasma [pyridoxal phosphate (PLP) > pyridoxic acid (PA) > pyridoxal] differed from that of CSF (pyridoxal > PLP > PA > pyridoxamine). Sex influenced vitamin B-6 vitamer concentrations in plasma and CSF and should therefore be taken into account when interpreting vitamin B-6 vitamer concentrations. The strict ratios and strong correlations between vitamin B-6 vitamers point to a tight regulation of vitamin B-6 vitamer concentrations in blood and CSF. Given the unique design of this study, with simultaneously withdrawn blood and CSF from a large number of subjects, reliable CSF:plasma ratios and correlations of vitamin B-6 vitamers could be established.

Conclusions: We provide an extensive reference set of vitamin B-6 vitamer concentrations in plasma and CSF. In addition to providing insight on the regulation of individual vitamers and their intercompartmental distribution, we anticipate that these data will prove to be a valuable reference set for the diagnosis and treatment of conditions associated with altered vitamin B-6 metabolism. Am J Clin Nutr 2014;100:587–92.

INTRODUCTION

Pyridoxal phosphate (PLP), the predominantly active form of vitamin B-6, is well known for its cofactor function in numerous enzymatic reactions in the central nervous system, where it mainly catalyzes amino acid and neurotransmitter metabolism. In addition, PLP is required for the actions of, among >160 other enzymes (1), glycogen phosphorylase (glucose biosynthesis), cystathionine β-synthase (homocysteine metabolism), and aminovalenilate synthase (heme biosynthesis). PLP-dependent enzymes also play an important role in the synthesis of neuroprotective compounds in the brain, such as kynurenic acid—an intermediate in the degradation pathway of tryptophan (2).

Inverse relations of vitamin B-6 with oxidative stress (3–5), inflammation (6–10), cardiovascular disease (11), diabetes (12), and cancer (13–22) have been reported. A higher intake of vitamin B-6 is associated with a lower risk of colorectal cancer (13, 19, 22) and breast cancer (14, 21), whereas higher concentrations of serum PLP are associated with a lower risk of lung cancer (18). Lower concentrations of plasma PLP have been associated with poorer cognition (23, 24).

The recent elucidation of these multiple roles for vitamin B-6, and the discovery of inborn errors of metabolism resulting in functional vitamin B-6 deficiency, have raised scientific interest in vitamin B-6. We hypothesize that, with direct analysis of vitamin B-6 in body fluids, functional vitamin B-6 deficiency can be reliably diagnosed, and the biochemical effects of treatment with vitamin B-6 can be monitored. This will not only increase our insight on vitamin B-6 vitamer concentrations in health and disease, but will also deepen our understanding of human vitamin B-6 metabolism and transport. It is essential to study concentrations of all vitamin B-6 vitamers in plasma and cerebrospinal fluid (CSF) (25, 26) and, for optimal comparison between plasma and CSF, both body fluids should be obtained simultaneously from the same individual.

In the literature, concentrations of the vitamin B-6 vitamers pyridoxine, pyridoxamine, pyridoxamine phosphate (PMP),...
pyridoxal, PLP, and the degradation product of vitamin B-6, pyridoxic acid (PA) have been reported for plasma. In most publications (26–35), PLP, PA, and pyridoxal were reported to be the vitamin B-6 vitamers most abundantly present in plasma (see Supplementary Table 1 under “Supplemental data” in the online issue). In the CSF of newborn infants (36) and children (37), pyridoxal was reported to be the most abundant vitamin B-6 vitamer, followed by PLP (see Supplementary Table 2 under “Supplemental data” in the online issue).

In the current study, we measured concentrations of pyridoxine, pyridoxamine, PMP, pyridoxal, PLP, and PA in plasma and the CSF of a large number of adult subjects. We studied the relation between vitamin B-6 vitamers in plasma and the CSF and between plasma and CSF and we investigated the possible influence of sex.

**SUBJECTS AND METHODS**

**Subjects and sample collection**

Of 533 healthy adult subjects (18–63 y of age), fasting plasma and CSF were collected. The first participant was recruited before July 2008, and plasma and CSF were collected preceding the administration of spinal anesthesia for minor elective surgery at different hospitals in and near Utrecht, Netherlands, between July 2008 and November 2011. Subject characteristics and details of sample collection were described previously (38–40). In summary, subjects were included if they were of North-Western European descent (ie, all grandparents born in the Netherlands, Belgium, France, Germany, Denmark, or the United Kingdom). Subjects with a history of self-reported psychotic or major neurological disorders (stroke, brain tumors, and neurodegenerative disease) were excluded.

**Sample storage**

After withdrawal, plasma and CSF samples were stored at −80°C and protected from light until further analysis. To study the influence of temperature on vitamin B-6 vitamer concentrations in CSF, aliquots stored at −20°C were compared with aliquots stored at −80°C (n = 62), because it was previously shown that vitamin B-6 vitamers are stable when samples are stored at −80°C (28, 34).

**Quantification of vitamin B-6 vitamers**

Concentrations of pyridoxine, pyridoxamine, PMP, pyridoxal, PLP, and PA were measured in plasma and CSF by ultra HPLC–tandem mass spectrometry according to the method of van der Ham et al (37). This method, developed for the analysis of CSF, was adapted for application in plasma.

For the measurement of vitamin B-6 vitamer concentrations in plasma, 100 μL plasma and internal standards was used. Samples were centrifuged twice after protein precipitation with trichloroacetic acid (5 min, 13,000 rpm). Calibration curve end concentrations of pyridoxal, PLP, and PA were adjusted for quantification of these vitamin B-6 vitamers in plasma (160, 200, and 185 nmol/L, respectively). For the quality-control (QC) samples, the plasma of random subjects was pooled, and vitamin B-6 vitamers were spiked to achieve 3 different concentrations (QC1–3). QC1–3 were used to study interassay variations (n = 10) for the different vitamin B-6 vitamers. Limits of detection and quantification were determined by using QC1 (n = 10; signal-to-noise ratios of 3 and 10, respectively) (see Supplementary Table 3 under “Supplemental data” in the online issue). PMP in plasma was not detectable because of instability.

**Statistical analysis**

SPSS 20.0 (IBM Corporation) was used for the statistical analysis. Because none of the vitamin B-6 vitamers in plasma and CSF, nor their unstandardized residuals, showed a normal distribution, nonparametric (Mann-Whitney U) tests were applied to study differences in vitamin B-6 vitamer concentrations, and 95% CIs were calculated for median vitamin B-6 vitamer concentrations and their 2.5th and 97.5th percentiles by using bootstrap analysis. Spearman’s ρ was used to describe correlations.

**RESULTS**

In plasma and CSF, pyridoxal, PLP, and PA were present at levels above the limit of quantification (LOQ) of the analysis method used. Pyridoxamine was present in quantifiable amounts in CSF only; in plasma it was below the LOQ. PMP was <LOQ in CSF (37), and in plasma it was highly instable; therefore, no reliable results could be obtained. Pyridoxine was <LOQ in both CSF (37) and plasma (<0.03 and <0.28 nmol/L, respectively; see Supplementary Table 3 under “Supplemental data” in the online issue).

Ten of 533 subjects with one or more extremely low or high vitamin B-6 vitamer concentrations in plasma or CSF were excluded from the reference set. In 5 of the 10 excluded subjects, pyridoxine was present in the CSF (0.5–125 nmol/L) and/or plasma (0.7–2.1 nmol/L), which points to vitamin B-6 supplementation (26, 37). In the other 5 subjects, concentrations of one or more vitamin B-6 vitamers were >1.5 times lower or higher than the lower or upper reference limit. As a result, vitamin B-6 vitamers of 523 subjects (plasma, n = 502; CSF, n = 424 and both n = 404) were further analyzed, and concentrations of pyridoxamine (in CSF), pyridoxal, PLP, and PA (in plasma and CSF) were studied in more detail.

**Vitamin B-6 vitamer concentrations in plasma and CSF**

The median concentrations of the different vitamin B-6 vitamers in plasma and CSF (nmol/L), and their respective ranges and 2.5th–97.5th percentile ranges, are shown in Table 1. The most abundant vitamin B-6 vitamer in plasma was PLP [median concentration 55.9 (2.5th–97.5th percentile range, 19.8–200) nmol/L], whereas in CSF the concentration of pyridoxal [median concentration 30.0 (2.5th–97.5th percentile range, 17.4–54.5) nmol/L] was the highest. Vitamin B-6 vitamer concentrations in plasma and CSF did not correlate with age (data not shown).

**Influence of storage temperature**

PLP, pyridoxamine, and pyridoxal in CSF were not stable during storage at −20°C. Concentrations of PLP and pyridoxal decreased with time and became undetectable after 10 and 20 mo, respectively. On the contrary, concentrations of pyridoxamine increased up to 500% in 15 mo.

**Influence of sex**

In plasma and CSF, concentrations of both pyridoxal and PLP were influenced by sex. The median concentration of pyridoxal in
CSF was lower in men (29.5 (2.5th–97.5th percentile range, 16.8–56.3) nmol/L; \( P = 0.022 \)) compared with 32.1 (2.5th–97.5th percentile range, 17.3–55.1) nmol/L; whereas the median concentration of pyridoxal in plasma was higher in men (11.0 (2.5th–97.5th percentile range, 4.2–25.5) nmol/L; \( P = 0.001 \)) compared with 9.2 (2.5th–97.5th percentile range, 3.9–24.4) nmol/L.

Median concentrations of PLP in both plasma and CSF were higher in men than in women (60.2 (2.5th–97.5th percentile range, 23.5–208) compared with 44.0 (2.5th–97.5th percentile range, 16.0–167) nmol/L for plasma; \( P < 0.001 \); 17.0 (2.5th–97.5th percentile range, 7.4–35.2) compared with 14.0 (2.5th–97.5th percentile range, 6.4–34.4) nmol/L for CSF; \( P < 0.001 \)). Concentrations of the other vitamin B-6 vitamers in plasma and CSF did not differ between men and women (data not shown).

### Vitamin B-6 vitamer ratios and correlations in and between plasma and CSF

The ratios and correlations between pyridoxamine, PL, PLP, and PA in plasma and CSF are shown in Table 2. In plasma, the strongest correlation was observed between PLP and pyridoxal (\( r = 0.564, P < 0.001; \) Figure 1A). In CSF, concentrations of PA and pyridoxal were correlated (\( r = 0.536, P < 0.001 \)).

In Table 3, ratios and correlations are shown for pyridoxal, PLP, and PA between CSF and plasma. Strong correlations between concentrations in CSF and plasma were observed for all

### TABLE 1

Concentrations of PM, PL, PLP, and PA in the plasma and CSF of adult subjects (18–63 y; \( n = 523 \))

<table>
<thead>
<tr>
<th>Vitamin B-6 vitamer concentration and body fluid</th>
<th>Median (95% CI)</th>
<th>Range</th>
<th>2.5th percentile (95% CI)</th>
<th>97.5th percentile (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>0.36 (0.34, 0.38)</td>
<td>&lt;0.04 (^2) to 1.2</td>
<td>0.06 (0.04 (^2), 0.09)</td>
<td>0.9 (0.8, 1.0)</td>
</tr>
<tr>
<td>PL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>10.5 (10.2, 10.9)</td>
<td>3.0–56.2</td>
<td>4.2 (3.7, 4.5)</td>
<td>24.5 (20.8, 33.0)</td>
</tr>
<tr>
<td>CSF</td>
<td>30.0 (29.1, 31.0)</td>
<td>13.5–78.5</td>
<td>17.4 (15.5, 18.4)</td>
<td>54.5 (49.3, 61.9)</td>
</tr>
<tr>
<td>PLP</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Plasma</td>
<td>55.9 (52.3, 59.1)</td>
<td>10.2–335</td>
<td>19.8 (18.0, 21.9)</td>
<td>200 (162, 217)</td>
</tr>
<tr>
<td>CSF</td>
<td>16.1 (15.2, 16.8)</td>
<td>5.3–49.2</td>
<td>6.9 (6.4, 7.7)</td>
<td>34.0 (29.3, 37.1)</td>
</tr>
<tr>
<td>PA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>23.6 (22.2, 25.0)</td>
<td>2.7–243</td>
<td>6.1 (5.1, 6.8)</td>
<td>107 (85.3, 134)</td>
</tr>
<tr>
<td>CSF</td>
<td>1.15 (1.04, 1.25)</td>
<td>&lt;0.09 (^2) to 8.7</td>
<td>&lt;0.09 (^2)</td>
<td>3.8 (3.1, 5.4)</td>
</tr>
</tbody>
</table>

\(^1\) CSF, cerebrospinal fluid; PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal phosphate; PM, pyridoxamine.

\(^2\) Limit of quantification of this vitamin B-6 vitamer (37).

\(^3\) Sex-related differences in concentrations of PL and PLP in plasma and CSF are provided elsewhere (see Supplementary Table 4 under “Supplemental data” in the online issue).

### TABLE 2

Ratios between PM, PL, PLP, and PA in the plasma (\( n = 502 \)) and CSF (\( n = 424 \))

<table>
<thead>
<tr>
<th>Vitamin B-6 vitamer ratio and body fluid (^2)</th>
<th>Median (95% CI)</th>
<th>Range</th>
<th>2.5th percentile (95% CI)</th>
<th>97.5th percentile (95% CI)</th>
<th>Correlation (( \rho )) (^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL:PM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>87.1 (78.3, 96.1)</td>
<td>16.6–774</td>
<td>29.1 (24.6, 35.6)</td>
<td>446 (347, 594)</td>
<td>0.154</td>
</tr>
<tr>
<td>PL:PL</td>
<td></td>
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</tr>
<tr>
<td>Plasma</td>
<td>5.4 (5.2, 5.7)</td>
<td>1.1–36.8</td>
<td>2.3 (1.9, 2.6)</td>
<td>13.7 (12.6, 16.1)</td>
<td>0.564**</td>
</tr>
<tr>
<td>CSF</td>
<td>0.5 (0.5, 0.6)</td>
<td>0.2–1.8</td>
<td>0.2 (0.2, 0.3)</td>
<td>1.1 (1.0, 1.2)</td>
<td>0.265*</td>
</tr>
<tr>
<td>PLP:PL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>50.0 (46.7, 54.8)</td>
<td>7.8–553</td>
<td>11.7 (9.9, 14.5)</td>
<td>274 (204, 316)</td>
<td>0.033</td>
</tr>
<tr>
<td>PA:PL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>2.4 (2.2, 2.5)</td>
<td>0.3–15.2</td>
<td>0.7 (0.5, 0.8)</td>
<td>7.8 (7.2, 8.7)</td>
<td>0.395*</td>
</tr>
<tr>
<td>CSF</td>
<td>0.04 (0.04, 0.04)</td>
<td>0.00–0.13</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.10 (0.09, 0.10)</td>
<td>0.536**</td>
</tr>
<tr>
<td>PA:PLP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>0.44 (0.41, 0.46)</td>
<td>0.03–2.5</td>
<td>0.12 (0.10, 0.14)</td>
<td>1.4 (1.2, 1.5)</td>
<td>0.414*</td>
</tr>
<tr>
<td>CSF</td>
<td>0.07 (0.06, 0.08)</td>
<td>0.00–0.38</td>
<td>0.01 (0.00, 0.01)</td>
<td>0.24 (0.20, 0.27)</td>
<td>0.198*</td>
</tr>
<tr>
<td>PA:PM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>3.2 (2.8, 3.8)</td>
<td>0.1–44.0</td>
<td>0.2 (0.2, 0.5)</td>
<td>16.8 (15.4, 21.8)</td>
<td>0.172*</td>
</tr>
</tbody>
</table>

\(^4\) Significant at \( P < 0.001 \). \(^5\) Significant at \( P < 0.001 \) and \( \rho > 0.500 \). CSF, cerebrospinal fluid; LOQ, limit of quantification; PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal phosphate; PM, pyridoxamine.

\(^2\) For ratio calculations, vitamin B-6 vitamer concentrations < LOQ were replaced by the determined LOQ of the respective vitamin B-6 vitamer (\( n = 6 \) for PM and \( n = 21 \) for PA).

\(^3\) Spearman’s \( \rho \).
Vitamin B-6 vitamers in human plasma and CSF

Humans depend on dietary sources of vitamin B-6, because we are unable to synthesize vitamin B-6. In our diet, various vitamin B-6 vitamers are present, which are converted into PLP. The vitamin B-6 vitamer composition of plasma (PLP > PA > pyridoxal) differs from that of CSF (pyridoxal > PLP > PA > pyridoxamine). In recent in vitro studies, we showed that the intestine plays an important role in the conversion of precursor vitamin B-6 vitamers (pyridoxine and pyridoxamine) into PLP and pyridoxal (41). Both uptake of vitamin B-6 from the diet and subsequent intestinal and hepatic metabolism result in PLP being the dominant vitamin B-6 vitamer in plasma. This is in contrast with CSF, where pyridoxal is most abundant. Pyridoxamine is not detectable in plasma, as was also previously reported (34, 35). Likewise, pyridoxine is not present in plasma or in CSF, unless subjects are supplemented with vitamin B-6 (26, 37).

In addition to the previously mentioned processes associated with vitamin B-6 status, altered vitamin B-6 vitamer concentrations can also be used in the diagnosis of functional vitamin B-6 deficiency, which can result from antinutritin deficiency [Online Mendelian Inheritance in Man (OMIM) 266100] (42), pyridox(am)ine-5'-phosphate oxidase (PNPO) deficiency (OMIM 610090) (25), hypophosphatasia (alkaline phosphatase deficiency; OMIM 241500) (43, 44) and hyperprolinemia type II (pyrroline-5-carboxylate dehydrogenase deficiency; OMIM 239510) (45). In addition, yet unknown causes of functional vitamin B-6 deficiency have been reported (25, 46–48). Patients present with convulsions and, frequently, developmental delay (49). Although treatment with vitamin B-6 (pyridoxine or PLP) is often successful in reducing convulsions, developmental delay still occurs (49, 50).

Indeed, decreased concentrations of PLP (25, 51, 52) and pyridoxal (25) have been found in the CSF of patients with PNPO deficiency and antinutritin deficiency [decreased PLP only (53)]. Concentrations of the other vitamin B-6 vitamers were not reported, whereas these might be abnormal as well and may have an effect on diagnosis and treatment. In plasma, vitamin B-6 vitamer concentrations have been published only for PNPO- and

![FIGURE 1. A: Correlation (Spearman’s ρ, P value) between PLP and PL in plasma (ρ = 0.564, P < 0.001; n = 502). B: Correlation (Spearman’s ρ, P value) between PLP in CSF and PLP in plasma (ρ = 0.629, P < 0.001; n = 404). CSF, cerebrospinal fluid; PL, pyridoxal; PLP, pyridoxal phosphate.](image)

**TABLE 3**

<table>
<thead>
<tr>
<th>Vitamin B-6 vitamer</th>
<th>Median (95% CI)</th>
<th>Range</th>
<th>2.5th percentile (95% CI)</th>
<th>97.5th percentile (95% CI)</th>
<th>Correlation (ρ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>2.9 (2.8, 3.1)</td>
<td>0.9–10.6</td>
<td>1.6 (1.4, 1.7)</td>
<td>7.2 (6.3, 7.6)</td>
<td>0.467*</td>
</tr>
<tr>
<td>PLP</td>
<td>0.3 (0.3, 0.3)</td>
<td>0.1–0.9</td>
<td>0.1 (0.1, 0.1)</td>
<td>0.7 (0.6, 0.8)</td>
<td>0.629**</td>
</tr>
<tr>
<td>PA</td>
<td>0.04 (0.04, 0.05)</td>
<td>0.00–0.35</td>
<td>0.00 (0.00, 0.01)</td>
<td>0.17 (0.13, 0.22)</td>
<td>0.486*</td>
</tr>
</tbody>
</table>

*Significant at P < 0.001. **Significant at P < 0.001 and ρ > 0.500. CSF, cerebrospinal fluid; LOQ, limit of quantification; PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal phosphate.

For ratio calculations, vitamin B-6 vitamer concentrations < LOQ were replaced by the determined LOQ of the respective vitamin B-6 vitamer (n = 21 for PA).
antiquitin-deficient patients receiving vitamin B-6 supplementation (26). Indirectly, altered vitamin B-6 vitamer concentrations can also reflect riboflavin status, because the PNPO enzyme requires flavin mononucleotide as an indispensable cofactor (54).

Vitamin B-6 metabolism

The strict ratios and strong correlations between PLP and pyridoxal in plasma and between PA and pyridoxal in CSF suggest that concentrations of these vitamin B-6 vitamers are tightly regulated. Disturbances of these vitamin B-6 vitamer ratios in plasma and CSF may therefore indicate possible deficiencies of the enzymes involved in vitamin B-6 metabolism: pyridoxal kinase (which phosphorylates pyridoxal into PLP) and pyridoxal phosphatase [which hydrolyzes PLP into pyridoxal (55)] and pyridoxal oxidase, which is involved in the degradation of pyridoxal into PA (56). It is therefore relevant to determine concentrations of all vitamin B-6 vitamers when investigating possible vitamin B-6–related disease (see Supplementary Table 5 under “Supplemental data” in the online issue for ratios and correlations between vitamin B-6 vitamers as reported in literature).

Vitamin B-6 transport

Little is known about the mechanism by which any of the vitamin B-6 vitamers is transported from blood to brain. At a biochemical level, there is evidence for carrier-mediated transport in the choroid plexus and blood-brain barrier (57), but a vitamin B-6 transporter protein has not yet been characterized. The strong correlations for pyridoxal and PLP between CSF and plasma may reflect transport at the blood-brain barrier or choroid plexus. Disturbances of vitamin B-6 vitamer ratios between CSF and plasma may therefore point toward a problem in vitamin B-6 transport, in a way similar to the decreased CSF:plasma ratio of glucose that is found in GLUT1 (blood-brain barrier glucose transporter) deficiency (OMIM 606777). We therefore advocate to not only analyze vitamin B-6 vitamers in plasma or CSF, but in both body fluids simultaneously when investigating a functional vitamin B-6 deficiency of unknown cause.

Conclusion

With this study, we provide an extensive reference set of vitamin B-6 vitamer concentrations in the plasma and CSF of adult subjects. Our data suggest a tight regulation of vitamin B-6 vitamers in and between blood and CSF. For adequate interpretation of vitamin B-6 vitamer concentrations, the influence of sex should be taken into account and samples should be stored adequately. In addition to providing insight on the regulation of individual vitamers and their intercompartmental distribution, we anticipate that these data will prove to be a valuable reference set for the diagnosis and treatment of conditions associated with altered vitamin B-6 metabolism.

We thank Jacobine E Buizer-Voskamp for her coordinational support and are grateful to Teus H Kappen for providing some of the plasma and CSF samples.

The authors’ responsibilities were as follows—MA, JJJ, SCB, ES, PJB, PJMK, EPAvD, PB, GV, RAO, and NMV-D: designed the research; MA, MB, JJMI, JJJL, SCB, ES, PJB, PJMK, EPAvD, PB, MGMDs-vdV, GV, NVVAMK, RAO, and NMV-D: conducted the research and wrote the manuscript; MA, MB, JJMJ, MGMDs-vdV, GV, and NMV-D: analyzed the data; and NMV-D: had primary responsibility for the final content. All authors read and approved the final manuscript. None of the authors declared any conflicts of interest.

REFERENCES


