Pyridoxine-Related Metabolite Concentrations in Normal and Down Syndrome Amniotic Fluid

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Introduction

In the past, some have espoused vitamin supplementa-tion regimes to treat postnatal Down syndrome. Most au-thorities consider these treatments unproven, if not dis-proven. In contrast, there is some literature on the bio-chemistry of pyridoxine in Down syndrome. The present study is intended to address the same biochemical ques-tions in the fetus that were previously addressed in post-natal children.

As reviewed by Pueschel [1], a number of studies have been conducted on the biochemistry of pyridoxine-re-lated metabolites and interactions with tryptophan me-tabolism in Down syndrome. Some are briefly reviewed here to address the clinical relevance of the present study. Coburn and Seidenberg [2] found reduced levels of pyri-doinal-5-phosphate in Down syndrome leukocytes. In a double-blind study, Pueschel et al. [3] reported that 5-hy-droxyindole levels were elevated after treatment with ei-ther 5-hydroxytryptophan or pyridoxine. Treatment with 5-hydroxytryptophan was not pursued because it was accompanied by infantile spasms in some cases [4]. In children with Down syndrome, the amplitude of corti-
cal auditory-evoked responses is high compared with normal children. Chronic treatment of 3-year-olds with pyridoxine appeared to decrease the cortical auditory-evoked potentials to the normal range [5, 6]. The relationship between pyridoxine and cortical auditory-evoked potentials was not stated. Pyridoxine (vitamin B6) increased 5-hydroxytryptophan levels, confirming similar findings of earlier reports [5, 6].

McCoy et al. [7] conducted a series of experiments on children with Down syndrome involving a pyridoxine antagonist and tryptophan loading test. McCoy and his group measured oxalate, xanthurenate and kynurenine as markers of pyridoxine deficiency. They found levels of oxalate in children with Down syndrome to be higher compared with normal children; findings for xanthurenate and kynurenine were unconvincing. The hypothesis of the current study was that abnormalities of pyridoxine-related metabolites would be seen in the amniotic fluid from fetuses with Down syndrome when compared with fluid from normal fetuses. The present study attempts to extend McCoy et al.’s [7] work to amniotic fluid and thus confronts the question: does the same biochemistry apply to both fetuses and infants with Down syndrome?

**Materials and Methods**

Archived amniotic fluid specimens from normal fetuses and fetuses with Down syndrome were obtained from the cytogenetic laboratory at the Medical College of Virginia in Richmond, Va., USA. The abnormals had been collected from 1993 to 1995, and the normals in 1996. Since old normal specimens had been discarded, normals from 1993 were not available. All specimens were stored in the same manner at –20°C, which is standard temperature for long-term freezing of metabolic specimens. Since this is well below the freezing point of water, oxidation of metabolites is unlikely. The median gestational age of the control specimens was 15.7 weeks and the median gestational age of the trisomic specimens was 16.7 weeks. This difference, while not zero, does not significantly affect the results.

Specimens were shipped on dry ice to Saint Louis University, Saint Louis, Mo., USA, for metabolic analysis. The samples were prepared and analyzed as previously described for urine [8]. All of the metabolites were derivatized with a trimethylsilyl-derivating agent, then analyzed by gas chromatography/mass spectrometry. Data (in μM) obtained from 22 Down syndrome and 41 normal specimens was analyzed parametrically, with means and standard errors. Simple t test was employed to compare the two groups where p was less than 0.05.

The protocol for this study was submitted to the institutional review boards at the Medical College of Virginia and Saint Louis University, both of whom waived further review since the work involved the use of archived specimens.

**Results**

The level of oxalate was found to be nearly 3 times higher in Down syndrome amniotic fluid when compared to normal, and this result was statistically significant (p = 0.0038) (table 1). Levels of other pyridoxine-related metabolites, 4-pyridoxic acid, kynurenine and xanthurenate did not significantly differ between amniotic fluids from normal and from Down syndrome groups. Simple t test was employed to compare the two groups where p was less than 0.05.

**Discussion**

Some biochemical markers which can be used in pyridoxine deficiency include xanthurenic acid and kynurenine. These were not significantly different between normal and Down syndrome fetuses. 4-Pyridoxic acid is a marker of pyridoxine supplementation. Again there was no statistically significant difference. These findings would suggest that there was no classic deficiency of pyridoxine in fetuses with Down syndrome.

These results are consistent with the work of McCoy et al. [7] who required a pyridoxine antagonist and tryptophan loading in order to demonstrate differences with xanthurenic, kynurenine and 4-pyridoxic acid in chil-

| Table 1. Pyridoxine-related metabolites (μM) in Down syndrome and normal amniotic fluid |

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Down mean</th>
<th>Standard error</th>
<th>Normal mean</th>
<th>Standard error</th>
<th>p (pooled)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalate</td>
<td>36.477</td>
<td>8.917</td>
<td>12.634</td>
<td>3.311</td>
<td>0.0038*</td>
</tr>
<tr>
<td>4-Pyridoxic acid</td>
<td>1.084</td>
<td>0.224</td>
<td>1.257</td>
<td>0.282</td>
<td>NS</td>
</tr>
<tr>
<td>Kynurenine</td>
<td>1.180</td>
<td>0.236</td>
<td>1.178</td>
<td>0.259</td>
<td>0.039</td>
</tr>
<tr>
<td>Xanthurenate</td>
<td>0.005</td>
<td>0.003</td>
<td>0.039</td>
<td>0.034</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Statistically significant (p ≤ 0.05).
Use of pyridoxine antagonist and tryptophan loading are provocative techniques employed to detect a slight pyridoxine deficiency. McCoy et al. found that xanthurenone and kynurenine did not differ between normal children and children with Down syndrome unless provocative techniques were used. It is not surprising that no differences were found with these metabolites in our study, since no pyridoxine antagonist was used and no tryptophan loading tests were performed. The current results, concerning the B$_6$-related metabolites xanthurenone, kynurenine and 4-pyridoxic acid in amniotic fluid, are thus consistent with those of McCoy et al. [7].

While xanthurenone and kynurenine are considered the classic biochemical markers of B$_6$ deficiency, one could consider other metabolites as part of a broader realm of B$_6$-related metabolites. In the current data, statistically significant elevation of oxalate was found in fetuses with Down syndrome. Oxalate may behave as if it were a marker of pyridoxine (vitamin B$_6$) deficiency.

According to a mechanism proposed by Nath et al. [9], deficiency of pyridoxine impairs transaminase function, including glycine-glyoxylate transaminase, thereby increasing the glyoxylate pool. Oxalate elevation may result from pyridoxine deficiency, and oxalate falls with pyridoxine supplementation [9]. Oxalate is also clinically relevant as a component of calcium oxalate kidney stones. Therapy with either B$_6$ [10] or B$_6$ plus magnesium [11] may suppress oxalate and reduce the recurrence of kidney stones. While it may not be one of the two classical markers, oxalate indeed functions, as it were, as a marker of pyridoxine deficiency.

The oxalate results in table 1 are consistent with a subtle abnormality of pyridoxine metabolism in fetuses with Down syndrome compared with normal fetuses. It remains unclear why one marker of pyridoxine deficiency, oxalate, would be elevated. It is noteworthy that these results are consistent with the study by McCoy et al. [7] on the urine of infants.

This is the first paper to elucidate such a finding in fetuses. In a future effort, the use of stable isotope dilution techniques, marker metabolites and selective ion monitoring would allow more careful quantitative assessment of these metabolites. However, these techniques would also increase the difficulty and expense of the study.

There are other factors for consideration in future studies. Since oxalate is also known to come from dietary sources and from microbial metabolism, these might be confounding factors. Gestational age should also be considered. Metabolites in amniotic fluid tend to fall with advancing gestation. Gestational age could not explain elevated oxalate in Down syndrome fetuses, as the gestational age in the Down syndrome group (16.7 weeks) was greater than the gestational age in the control group (15.7 weeks).

**Conclusion**

The methods described here would not be recommended for prenatal diagnosis. Prenatal diagnosis of inborn errors of metabolism is accurately done using a stable isotope dilution method, marker metabolites and selective ion monitoring. Prenatal diagnosis of chromosomal disorders is accurately done by chromosomal analysis and/or by fluorescence in situ hybridization. If the methods described in this paper were used for prenatal diagnosis, it is likely that some fetuses would be misdiagnosed. The goal of this study is to further our understanding of the pathophysiology and biochemistry of Down syndrome.

Over the past several decades, the health and longevity of patients with Down syndrome has improved. This improvement has not come from the discovery of a single magic bullet. Rather, it comes from the identification of many of the problems that these children have and their treatment. One example would be the testing of trisomic children for hypothyroidism and their treatment with thyroxine. It is hoped that with careful scientific study, greater understanding of fetal Down syndrome will lead to an improved quality of life for trisomic children.

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**References**


