

## Kynurenine pathway metabolites: relevant to vitamin B-6 deficiency and beyond<sup>1–3</sup>

Matthew A Ciorba

Pyridoxal 5' phosphate (PLP)<sup>4</sup>, the active form of vitamin B-6, is involved in a wide variety of physiologic processes including gluconeogenesis and the synthesis of sphingolipids and neurotransmitters. It also functions as a cofactor for many enzymes required for amino acid metabolism. Vitamin B-6 can be acquired from both animal and plant foods. It is also available through fortified cereals and supplements. Clinically, signs of isolated B-6 deficiency are rare. However, subclinical deficiency can precipitate biochemical changes that become more obvious as deficiency progresses.

An important biochemical change associated with vitamin B-6 deficiency is that of hyperhomocysteinemia (1). It was the recognition of hyperhomocysteinemia as a cardiovascular event risk factor that led to several trials aimed at assessing the clinical impact of supplementation with vitamin B-6, as well as folate and vitamin B-12. One such trial, conducted in Norway, was designed to examine these vitamins for secondary prevention of cardiovascular events and reduction in mortality (2). Although the trial ultimately showed no benefit from the supplements, the rich data set collected from >3000 enrolled adults has been useful in addressing additional questions about vitamin status.

In this issue of the Journal, Ulvik et al (3) use these data to identify a new serum marker for examining functional vitamin B-6 status. To achieve this, they measured several metabolites in the tryptophan-to-kynurenine pathway. As shown in their Figure 1, tryptophan is metabolized to kynurenine via either indoleamine 2,3 dioxygenase (IDO1 or IDO2) or tryptophan 2,3 dioxygenase (TDO). Other than 3-hydroxykynurenine (HK), all subsequent metabolites require a vitamin B-6 (PLP)-dependent enzyme for generation. They show that ratios of HK to metabolites downstream of PLP-dependent enzymes [xanthuranic acid (XA), 3-hydroxylantranilic acid (HAA), and kynurenic acid] correlate with PLP concentrations better than HK alone. The relation was strongest at the lowest PLP concentrations, and importantly, the ratios normalized after vitamin B-6 supplementation was instituted.

It is intriguing to think that plasma HK:XA and HK:HAA ratios reflect vitamin B-6 function in real-time physiology. Validation of this assay in a more diverse population would surely be needed before it could be adopted; even then, it would likely be most useful as a complementary test to the direct measurement of PLP concentrations. Still, there is much to be learned from this highly detailed and data-rich study. Along with the authors' pre-

vious work (4), this study provides insight as to the normal ranges of the entire panel of the kynurenine pathway metabolites and their ratios in a very large, albeit homogeneous, population. Moreover, important baseline population characteristics are provided, including inflammatory markers and smoking status.

These data are particularly relevant now as we begin to appreciate and explore the variety of important physiologic and pathophysiologic functions mediated by kynurenine metabolites. For example, the immunomodulatory capacities of IDO are in part driven by kynurenines directly suppressing inflammatory T cell responses (5). This same mechanism likely supports tumoral immune escape in IDO- and TDO-expressing cancers. Both kynurenine and quinolinic acid (another kynurenine metabolite downstream of PLP-dependent enzymes) directly support tumor cell proliferation by activating  $\beta$ -catenin signaling in colon tumors (6). This pathway also promotes tumor growth through activation of the human aryl hydrocarbon receptor in other tumor types (7). Kynurenine itself also has vasodilatory properties and perhaps acts as a compensatory mechanism in atherosclerotic coronary disease (8). Finally, several of the kynurenine metabolites have neuromodulator effects that are predicted to play important roles in both psychiatric and neurodegenerative disorders (9). It is worth noting that the signaling events for most of these processes are likely attributable to high local kynurenine metabolite concentrations, rather than to circulating concentrations as discussed in the current article.

The plasma kynurenine-to-tryptophan ratio (KTR) has also been widely examined as a biomarker of immune activation. This ratio reflects activity of IDO1, which is highly upregulated in response to interferon- $\gamma$  and expressed in several tissue types

<sup>1</sup> From the Division of Gastroenterology, Inflammatory Bowel Diseases Program, Washington University in St Louis, St Louis, MO.

<sup>2</sup> Supported by National Institute of Diabetes and Digestive and Kidney Diseases grant DK089016 (MAC) and National Institute of Allergy and Infectious Diseases grant 5U01AI095776-03 Sub no. 9006862 (MAC).

<sup>3</sup> Address correspondence to MA Ciorba, Division of Gastroenterology, Inflammatory Bowel Diseases Program, Washington University in St Louis, 660 South Euclid Avenue, Box 8124, St Louis, MO 63110. E-mail: mciiorba@dom.wustl.edu.

<sup>4</sup> Abbreviations used: HAA, hydroxylantranilic acid; HK, 3-hydroxykynurenine; IDO, indoleamine 2,3 dioxygenase; KTR, kynurenine-to-tryptophan ratio; PLP, pyridoxal 5' phosphate; TDO, tryptophan 2,3 dioxygenase; XA, xanthuranic acid.

First published online August 28, 2013; doi: 10.3945/ajcn.113.072025.

as well as in macrophages and dendritic cells. Measurement of the IDO1 substrate and its downstream product limits potential bias from variations in dietary tryptophan. For example, the KTR corresponds well with Crohn disease activity and positively correlates with both the erythrocyte sedimentation rate and C-reactive protein (10). Unbiased metabolomic studies have confirmed that intestinal inflammation leads to changes in several metabolites of the kynurenine pathway as detected in urine and serum (11). In the current study, the authors accounted for the KTR, C-reactive protein, and neopterin in their analysis. Patients with higher inflammatory markers tended to have higher HK concentrations. In turn, the HK-based ratios more strongly correlated to PLP concentrations in these patients.

Future investigations will clarify whether these kynurenine pathway metabolite ratios will be useful for measuring functional vitamin B-6 status in patients with subclinical deficiency. At least one recent study provides support for their conclusions with the use of a mathematical modeling approach (12). Future clinical studies seeking to address this same question will also hopefully include more heterogeneous populations and include patients with autoimmune/inflammatory conditions. Until that time, however, Ulvik et al have certainly given us new insight into the complex world of using metabolite ratios to understand the physiologic underpinnings of the kynurenine pathway.

The author had no conflicts of interest to report.

## REFERENCES

1. Ubbink JB, Vermaak WJ, van der Merwe A, Becker PJ. Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia. *Am J Clin Nutr* 1993;57:47–53.
2. Ebbing M, Bleie O, Ueland PM, Nordrehaug JE, Nilsen DW, Vollset SE, Refsum H, Pedersen E, Nygard O. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. *JAMA* 2008;300:795–804.
3. Ulvik A, Theofylaktopoulos D, Midttun Ø, Nygård O, Eussen SJPM, Ueland PM. Substrate product ratios of enzymes in the kynurenine pathway measured in plasma as indicators of functional vitamin B-6 status. *Am J Clin Nutr* 2013;98:934–40.
4. Midttun O, Ulvik A, Ringdal Pedersen E, Ebbing M, Bleie O, Schartum-Hansen H, Milsen RM, Nygard O, Ueland PM. Low plasma vitamin B-6 status affects metabolism through the kynurenine pathway in cardiovascular patients with systemic inflammation. *J Nutr* 2011;141:611–7.
5. Fallarino F, Grohmann U, Vacca C, Orabona C, Spreca A, Fioretti MC, Puccetti P. T cell apoptosis by kynurenines. *Adv Exp Med Biol* 2003;527:183–90.
6. Thaker AI, Rao MS, Bishnupuri KS, Kerr TA, Foster L, Marinshaw JM, Newberry RD, Stenson WF, Ciorba MA. IDO1 metabolites activate beta-catenin signaling to promote cancer cell proliferation and colon tumorigenesis in mice. *Gastroenterology* 2013;145(2):416–25.e4.
7. Opitz CA, Litzemberger UM, Sahn F, Ott M, Tritschler I, Trump S, Schumacher T, Jestaedt L, Schrenk D, Weller M, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* 2011;478:197–203.
8. Pedersen ER, Svingen GF, Schartum-Hansen H, Ueland PM, Ebbing M, Nordrehaug JE, Iglund J, Seifert R, Nilsen RM, Nygard O. Urinary excretion of kynurenine and tryptophan, cardiovascular events, and mortality after elective coronary angiography. *Eur Heart J* (Epub ahead of print 25 July 2013).
9. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci* 2012;13:465–77.
10. Gupta NK, Thaker AI, Kanuri N, Riehl TE, Rowley CW, Stenson WF, Ciorba MA. Serum analysis of tryptophan catabolism pathway: correlation with Crohn's disease activity. *Inflamm Bowel Dis* 2012;18:1214–20.
11. Ciorba MA. Indoleamine 2,3 dioxygenase in intestinal disease. *Curr Opin Gastroenterol* 2013;29:146–52.
12. Rios-Avila L, Nijhout HF, Reed MC, Sitren HS, Gregory JF III. A mathematical model of tryptophan metabolism via the kynurenine pathway provides insights into the effects of vitamin B-6 deficiency, tryptophan loading, and induction of tryptophan 2,3-dioxygenase on tryptophan metabolites. *J Nutr* 2013;143:1509–19.