

Kynurenines and Vitamin B6: Link between Diabetes and Depression.

Gregory Oxenkrug^{1*}, Rebecca Ratner¹, Paul Summergrad¹

1. Psychiatry and Inflammation Program, Department of Psychiatry, Tufts University School of Medicine and Tufts Medical Center, Boston MA, USA.

ABSTRACT

The increased association between depression and diabetes mellitus is generally acknowledged. Recent studies suggest that depression leads to diabetes. However, the underlying molecular mechanisms for this association remain unclear. Literature and our data indicate that inflammatory and/or stress factors in depression up-regulate tryptophan (TRP) conversion into kynurenine (KYN), a substrate for nicotinamide adenine dinucleotide (NAD) biosynthesis. Deficiency of vitamin B6, a co-factor of the key enzymes of KYN – NAD pathway, shunts KYN metabolism from formation of NAD towards production of xanthurenic (XA) and kynurenic (KYNA) acids. Human and experimental studies reveal that XA, KYNA and their metabolites interfere with production, release and biological activity of insulin. We propose that inflammation- and/or stress-induced up-regulation of TRP – KYN metabolism in combination with vitamin B6 deficiency is one of the mechanisms mediating increased risk of diabetes in depression. Consequently, monitoring formation of diabetogenic KYN derivatives might help to identify subjects-at-risk for the development of diabetes. Pharmacological down-regulation of the TRP – KYN – NAD pathway and maintenance of adequate vitamin B6 status might help to prevent the development of diabetes in depression and other conditions associated with inflammation/stress-induced excessive production of KYN and vitamin B6 deficiency, e.g., obesity, cardiovascular diseases, aging, menopause, pregnancy, and hepatitis C virus infection.

Corresponding author: Oxenkrug Gregory F. goxenkrug@tuftsmedicalcenter.org

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Introduction.

The increased association between depression and diabetes mellitus is generally acknowledged (1,2). Observation of 65% increase risk for development of (mostly type 2) diabetes in a prospective study of clinically depressed patients (3) supports the hypothesis that depression leads to diabetes (4). Molecular mechanisms that mediate the increased risk of diabetes in depression remain undetermined although some hypotheses discussed elsewhere (2). Current review focuses on tryptophan (TRP) – kynurenine (KYN) – nicotinamide adenine dinucleotide (NAD) metabolism in depression; the effects of vitamin B6 on KYN – NAD metabolism, and on the effects of KYN and some of its derivatives on production, release and biological activity of insulin. Literature and our data suggest that depression is associated with the increased production of KYN from TRP in response to activation of rate-limiting enzymes of the TRP – KYN pathway induced by pro-inflammatory cytokines and/or stress hormones, and with deficiency of vitamin B6, a co-factor to the key enzymes of KYN - NAD metabolism. These two conditions are necessary for the formation of diabetogenic KYN derivatives.

Tryptophan – Kynurenine Metabolic Pathway.

Tryptophan (TRP) is an essential (for humans) amino acid. About 5 % of non-protein routes of TRP metabolism is utilized for formation of methoxyindoles: serotonin, N-acetylserotonin and melatonin [5,6] (Fig.1).

The major non-protein route of TRP metabolism is formation of KYN, catalyzed by rate-limiting enzymes: *indoleamine 2,3-dioxygenase* (IDO) or *TRP 2,3-dioxygenase* (TDO) [6]. IDO is activated by pro-inflammatory factors, e.g., interferon-gamma (IFNG), tumor necrosis factor-alpha, IL-1 beta, and lipopolysaccharide, while TDO is inducible by stress hormones, e.g., cortisol, prolactin, and by substrate, TRP [5] .

Kynurenine – nicotinamide adenine dinucleotide metabolic pathway.

KYN is substrate for two post-KYN metabolic pathways:

- 1). Formation of kynurenic acid (KYNA), catalyzed by *KYN aminotransferases* (KAT) [7]. KYNA -is a NMDA [6] and α -7 Nicotinic Acetylcholine Receptors antagonist [7] and a precursor of quinaldic acid (QA) [8]; and
- 2). Formation of 3-hydroxyKYN (3-HK) catalyzed by *KYN 3-monoxygenase* [5,6].

3-HK is a substrate for two metabolic pathways:

- 1). Formation of nicotinamide adenine dinucleotide (NAD). The first step of the 3-HK – NAD pathway catalyzed by *kynureninase*; and
- 2). Formation of xanthurenic acid (XA), catalyzed by *3-HK-transaminase* [9]. XA is a precursor of 8-hydroxyquinaldic acid (8-HQ) [10].

Pyridoxal 5'-phosphate and KYN – NAD metabolic pathway.

Pyridoxal 5'-phosphate (P5P), an active form of vitamin B6, is a cofactor for >100 metabolic reactions, including key enzymes of post-KYN metabolism: *KYN 3-monoxygenase*, *KAT* and *kynureninase*, - the latter enzyme is particularly sensitive to dietary vitamin B-6 restriction [11]. Down-regulation of *kynureninase*, caused by P5P deficiency, shifts 3-HK metabolism from formation of NAD to production of XA and KYNA [12]. P5P deficiency combined with up-regulated TRP conversion to KYN leads to increased availability of 3-HK as substrate for formation of XA and 8-HQ, and increased availability of KYN as substrate for KYNA and QA in the cerebellum, corpus striatum, frontal cortex, and pons/medulla [13], blood [14] and peripheral organs [15 – 17], including pancreatic islets [15].

Vitamin B6 depletion results in drastic increase while vitamin B6 supplementation normalizes urinary 3-HK and XA after TRP load in cardiac [19] and obese [20] patients and rats [16]. The other consequence of P5P deficiency-induced down-regulation of *kynureninase* is the decreased formation of NAD that subsequently inhibits synthesis and secretion of insulin and triggers death of pancreatic beta-cells [21, 22]. Considering that NAD inhibits TDO, decreased formation of NAD caused

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by P5P deficiency might result in further activation of TDO and increased production of KYN [23].

Besides P5P deficiency, *kynureninase* might be inhibited by XA, thus sustaining the accumulation of 3-HK, KYNA, KYN and XA at the expense of NAD production [24]. Additionally, XA might perpetuate P5P deficiency by inhibiting pyridoxal kinase, the enzyme which catalyzes the formation of P5P from vitamin B6 [25].

Diabetogenic effects of KYN derivatives.

Xanthurenic acid. XA was the first KYN metabolite to be observed in the increased amounts in the urine samples of type 2 diabetes patients in comparison with in healthy subjects [26]. Recent study found the increased levels of XA precursors, KYN and 3-HK in serum samples of diabetic retinopathy patients [27]. XA induced experimental diabetes in rats [28].

The possible mechanisms mediating XA contribution to the development of diabetes are 1). Formation of chelate complexes with insulin (XA-In). As antigens, XA-In complexes are indistinguishable from insulin but have 49% lower activity than pure insulin [28]; 2). Formation of Zn⁺⁺-ions – insulin complexes in β -cells that exert toxic effect in isolated pancreatic islets [29, 30]; 3). Inhibition of insulin release from rat pancreas [15]; and 4). Induction of pathological apoptosis of pancreatic beta cells through caspase-3 dependent mechanism [31, 32].

Kynurenic acid. KYNA was found to be increased in urine of nonhuman primate and mouse models of type 2 diabetes mellitus in a recent metabolomic study [33], and in patients with diabetic retinopathy [27]. The possible mechanisms of diabetogenic effect of KYNA might be related to KYNA ability to block NMDA receptors. Thus, NMDA antagonist and pharmacological precursor of KYNA, 7-chlorokynurenic acid, [6] and NMDA antagonist, MK-801, negated the inhibition of glucose production by NMDA agonists injected into dorsal vagal complex in rodents [34].

In addition, XA, KYNA, and their derivatives, QA and 8-HQ, inhibit pro-insulin synthesis in isolated rat pancreatic islets [35]

Recent study revealed elevated expression of IDO in serum samples of diabetic retinopathy patients [27]. In the same vein, surplus dietary TRP, the substrate for formation of KYNA and XA, induced insulin resistance in pigs [36].

KYN/TRP Ratio and Neopterin as Clinical Markers of up-regulation of TRP – KYN pathway.

Plasma (serum) ratio of KYN to TRP (KTR) is a generally accepted clinical marker of IDO activity [14]. Considering that both IDO and TDO regulate the rate of TRP conversion into KYN [37], plasma concentrations of TRP and KYN might be affected by the activity of stress hormone inducible TDO as well. However, KTR does reflect IDO activity in conditions associated with inflammation (38).

Concurrently with induction of IDO, pro-inflammatory factors (e.g., IFNG, TNF-alpha, IL-1beta) induce guanosine triphosphate cyclohydrolase I (GTPCH), a rate-limiting enzyme in the biosynthesis of tetrahydrobiopterine (BH4), the obligatory cofactor of nitric oxide (NO) synthase (NOS) [39]. GTPCH catalyzes GTP conversion into 7,8-dihydroneopterin (BH2) [39]. Pro-inflammatory factors-induced activation of GTPCH results in increased formation of neopterin, a stable, water-soluble derivative of BH2 [39]. Therefore, inflammation increased formation of neopterin might be considered not simply a clinical marker of inflammation, but an indirect marker of IDO activation as well [35].

Blood neopterin levels correlate with KTR in healthy humans [40], and cardiovascular patients [14]. We found similar strong ($r=0.68$) and highly significant ($p<0.0001$) correlation between serum KTR ratio and neopterin in 80 hepatitis C virus (HCV) patients treated with interferon-alpha [unpublished data].

Kynurenine/tryptophan ratio, neopterin and P5P levels in depression and diabetes.

Diabetes. Clinical and experimental data suggest there is increased metabolism of TRP in diabetes, most likely,

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resulting from up-regulation of TRP – KYN pathway. Thus, impaired accumulation of TRP in the brain concomitantly with a much faster disappearance of the administered TRP from the bloodstream was observed in streptozotocin-diabetic rats after TRP load [41]. Similarly, post-loading levels of plasma TRP (but not of other large neutral amino acids) increased less in diabetic patients than in healthy controls. [42]

Recent studies reveal decreased plasma TRP concentrations and increased KYN and KTR in 21 hemodialysis patients with diabetes in comparison with 40 healthy controls patients. An increase in neopterin was correlated with KYN concentrations ($r = 0.393$, $p < 0.01$), suggesting that increased TRP degradation is a result of IDO activation [43]. In the same vein, neopterin but not C-reactive protein, an inflammation marker not related to IDO/GTPCH activation, increased in diabetic in comparison with non-diabetic patients with critical limb ischemia (44).

Decreased kynureninase activity was observed in liver and kidney of alloxan diabetic rabbits [45]. Additionally, XA was identified in pre-diabetes subjects [46].

Neopterin correlated with insulin resistance, an early event in the pathogenesis of type 2-diabetes, in Caucasian population [14,47,48]. We observed correlation between plasma neopterin concentrations and IR (HOMA-IR, $r=0.08$, $P < 0.03$), and P5P ($r = -0.13$, $P = 0.002$) in 592 adult (45–75 years of age) participants of community dwellers in the Boston Puerto Rican Health Study. The strongest ($r=0.15$) and most significant ($P < 0.0002$) correlation was recognized between HOMA-IR and neopterin/P5P ratio (a combined index of increased inflammation and P5P deficiency) [49]. Low plasma concentrations of P5P have been reported in conditions associated with increased fasting glucose and glycated hemoglobin [50].

These results are in line with our hypothesis that combined up-regulation of the TRP – KYN pathway (as indirectly assessed by neopterin concentrations) and P5P deficiency serves as one of the mechanisms promoting the development of diabetes in depression.

Depression. It was initially suggested in 1969 that stress-induced TDO activation shunts TRP metabolism

from formation of serotonin towards production of KYN in depression [34, 51]. The discovery of inflammation-inducible IDO added another mechanism of up-regulation of KYN formation from TRP in depression [52]. Association of depression with an increased production of cortisol [53] and inflammatory factors [54] is described elsewhere. Both IDO and TDO activation leads to the same major consequences: 1). Deficiency of formation of serotonin (and its metabolites, melatonin and N-acetylserotonin) contributing to insomnia, dysregulation of biological rhythms and impaired neurogenesis observed in depression [55-57]; 2). Up-regulated formation of KYN and its neuroactive derivatives which exert anxiogenic, pro-oxidative and cognitive impairment effects typical for depression [58,59] (Fig.2).

Increased plasma neopterin levels were reported in depressed patients, further supporting the notion of IDO activation in depression [60].

Low plasma concentrations of P5P have been reported in depression (61). An increase in KTR and a deficiency in vitamin B6 might explain the increased production of XA in depressed patients (62).

Hepatitis C Virus, Depression and Diabetes.

Depression is the often side-effect of interferon (IFN)-alpha administration to patients with hepatitis C virus (HCV) infection. There is a strong correlation between increased production of KYN (and its derivatives) and severity of IFN-alpha-induced depression (63). We found an increased frequency of the carriers of high producer (T) allele, of IFNG(+874) T/A gene, that encodes production of IFNG protein, among HCV patients suffering from depression as a side-effect of IFN-alpha treatment (64). Serum neopterin concentrations were higher in HCV versus the non-HCV patient population [65] and predicted resistance to IFN-alpha therapy [66]. We found strong ($r=0.68$) and significant ($p < 0.0001$) correlation between the serum KTR ratio and neopterin in 80 hepatitis C virus (HCV) patients treated with interferon-alpha [unpublished data].

Incidence of diabetes is higher among HCV patients than in non-HCV population [67]. HCV infection significantly

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lowers vitamin B6 levels [68]. IFN-alpha treatment was associated with increased risk of developing insulin resistance and higher incidence of type 2 diabetes in comparison with the groups of both non-viral chronic liver disease [69] and patients with chronic hepatitis B virus [70]. Moreover, antecedent HCV infection markedly increases the risk of developing diabetes in susceptible subjects, while even non-diabetic HCV patients have insulin resistance and specific defects in the insulin-signaling pathway [71]. These data are in line with the current hypothesis that increased risk of IR in HCV patients depends on a combination of inflammation (e.g., IFN γ)-triggered up-regulation of TRP – KYN metabolism with P5P deficiency-induced dysregulation of KYN – NAD metabolic pathway.

Therapeutic interventions. Current hypothesis suggests that prevention and treatment of diabetes in depression and other conditions associated with chronic stress or chronic Th-1 type inflammation should include the supporting of adequate vitamin B6 status [72] and pharmacological modifications of TRP – KYN metabolism, aimed at down-regulating the formation of diabetogenic KYN derivatives. The latter may be achieved by administration of IDO and TDO inhibitors. The known IDO inhibitor, 1-methyl-L-TRP [73], is not available for human use. There are two IDO inhibitors available for human use: minocycline, an antibiotic with anti-inflammatory action [74,75], and antidepressant, wellbutrin [76]. It is noteworthy that wellbutrin, contrary to tricyclic antidepressants, has a favorable metabolic profile [77]. The strongest IDO inhibitor is berberine, an isoquinoline alkaloid isolated from *Berberis aristata*, an herb widely used in Indian and Chinese systems of medicine [78]. Berberine exerts therapeutic potential in diabetic hamsters [79] and diabetic patients [80,81]. It is noteworthy that both berberine and minocycline prolonged life span and improved health span in the *Drosophila* model [82, 83]. Consistent with our hypothesis, is observation that vitamin B6 supplementation dose-dependently decreased insulin levels and improved insulin resistance in KK-A(y) mice, an animal model of obese, type 2 diabetes [84, 85]. Current hypothesis is in line with the previously published reports of the neuroprotective effect of vitamin B6 [86] and its contribution to regulation of

choline and docosahexaenoic acid concentrations [87,88].

Conclusions. Review of literature and our data suggest that one of the mechanisms for the increased incidence of diabetes in depressed subjects might be up-regulation of TRP – KYN metabolism in combination with P5P deficiency, resulting in excessive formation of diabetogenic KYN derivatives.

Monitoring of KYN/P5P status and formation of XA and KYNA in depressed patients might help to identify subjects-at-risk for the developing of diabetes.

Support for adequate B6 status [89] and inhibition of TRP- KYN metabolism might prevent development of diabetes associated with depression.

This proposed biochemical mechanism may explain the increased risk of diabetes not only in depression, but in other conditions associated with the combined up-regulation of KYN production and P5P deficiency, e.g., obesity, cardiovascular disorders [90], menopause, aging, HCV and treatment with IFN-alpha [91]. It is noteworthy that pro-inflammatory activity of adipokines [92] and KYN production in white adipose tissue [93] has been reported along with higher plasma KTR in obese, but not lean, subjects [94].

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Fig.1. Vitamin B6 deficiency-induced shift of post-KYN metabolism from biosynthesis of NAD towards formation of diabetogenic KYN derivatives. **Abbreviations.** TRP – tryptophan; IFNG – interferon-gamma; IDO – indoleamine 2,3-dioxygenase; KYN – kynurenine; KMO – KYN-3-monooxygenase; 3-HK – 3-hydroxyKYN; P5P – pyridoxal 5'-phosphate; QUIN – quinolinic acid; NAD – nicotinamide adenine dinucleotide; KYNA – kynurenic acid; XA – xanthurenic acid; QA - quinaldic acid; 8-HQ – 8-hydroxyquinaldic acid; GTP – guanosine triphosphate; GTPCH – GTP cyclohydrolase I; BH2 - 7,8-dihydroneopterin;. BH4 – tetrahydrobiopterin; NOS – nitric oxide synthase

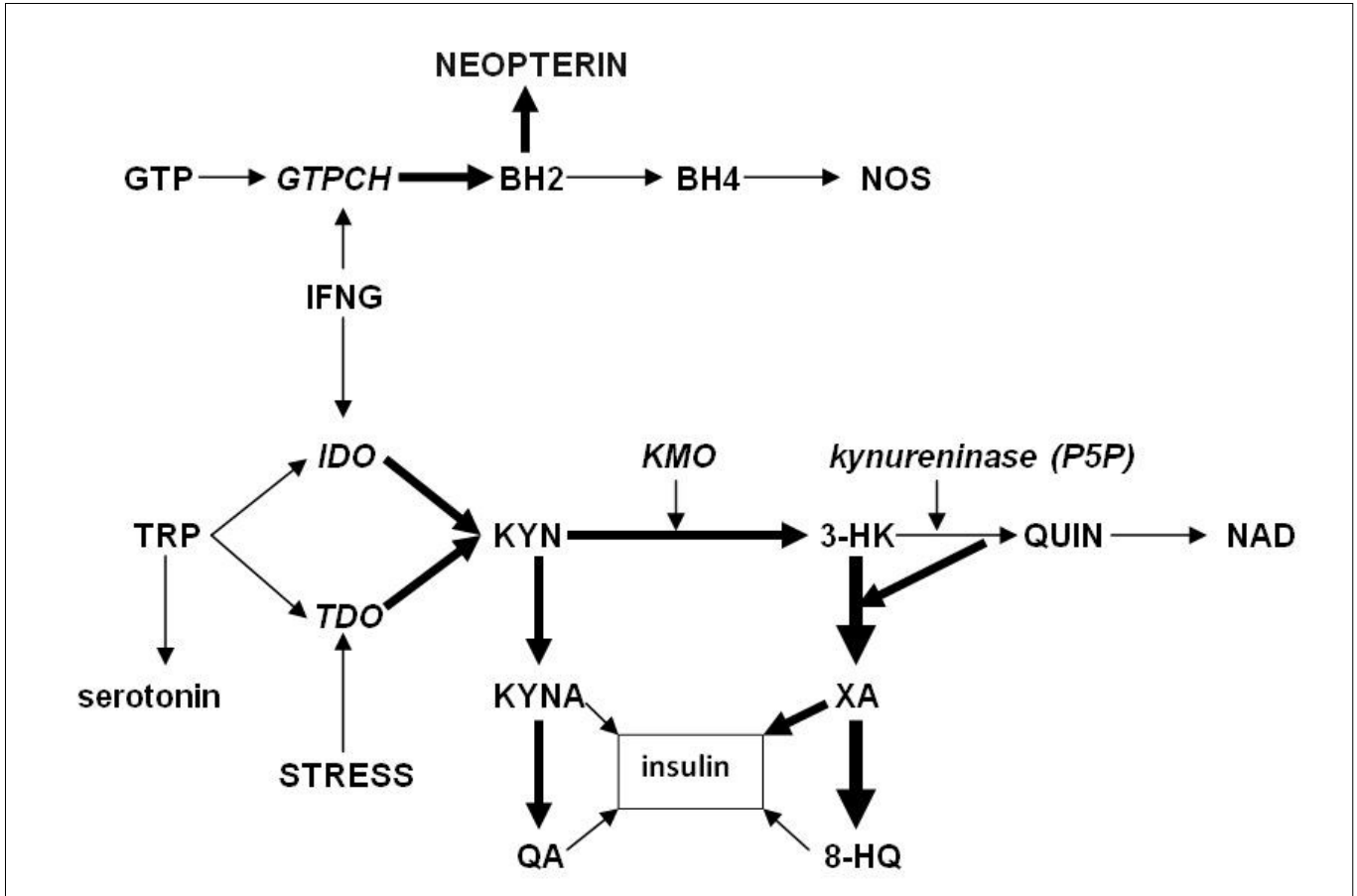
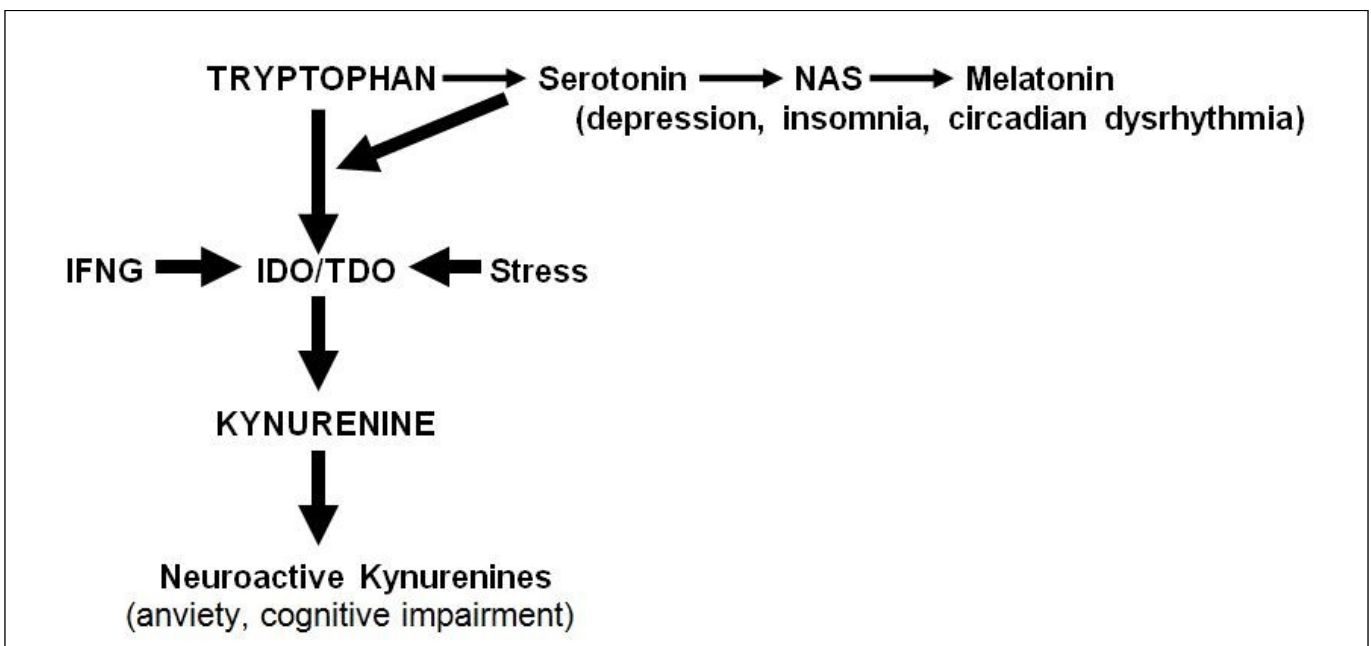


Figure 2. Shift of tryptophan metabolism in depression. **Abbreviations:** IFNG – interferon-gamma; NAS – N-acetylserotonin, IDO - indoleamine 2,3-dioxygenase, TDO – tryptophan 2,3-dioxygenase.



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Conflict of Interest Disclosure.

Paul Summergrad is a non-promotional speaker for CME outfitters, Inc., and consultant and non-promotional speaker for Pri-med, Inc.

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