The Metabolites in the Tryptophan Degradation Pathway Might Be Useful to Determine the Tolerable Upper Intake Level of Tryptophan Intake in Rats1–3

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Abstract

L-Tryptophan (L-Trp) is a rate-limiting amino acid for growth in people from underdeveloped countries. Because L-Trp is also a precursor to nicotinamide, administration of the free form of L-Trp is a very good method of preventing pellagra. Furthermore, L-Trp has shown some effectiveness for the treatment of a variety of other conditions typically associated with low serotonin levels in the brain. Therefore, information about the no-observed-adverse-effect level and lowest-observed-adverse-effect level of L-Trp is needed. However, it is not possible to experimentally obtain such data in humans due to ethical considerations. The aim of the present workshop was to identify biomarkers that could be used before the appearance of adverse effects of L-Trp. We reviewed the published research using rats to develop an index of the metabolic upper intake level for L-Trp to be used instead of the tolerable upper intake level (UL). These results show that the urinary excretory ratio of anthranilic acid:kynurenic acid is potentially the most sensitive and appropriate surrogate breakpoint index to predict the UL of L-Trp. J. Nutr. 142: 2227S–2230S, 2012.

Introduction

L-Tryptophan (L-Trp) is one of the limiting amino acids in people relying on cereals as the main source of protein, and fortification of cereals with the free form of L-Trp is a very good method for improving the amino acid nutritional status. The free form of L-Trp is also a very good precursor of nicotinamide (Nam)4 compared with the bound form of L-Trp.

About 20 y ago, a large outbreak of eosinophilia-myalgia syndrome occurred in individuals taking an L-Trp supplement produced by a single manufacturer (1,2). Although the component (s) in the supplement responsible for causing eosinophilia-myalgia syndrome has not been definitively established, this event stimulated interest in establishing a tolerable upper intake level (UL) for L-Trp. Considering the ethical problems with testing high doses of L-Trp in humans, the establishment of a biomarker for high intakes of L-Trp in experimental animals would be a useful step in establishing safety margins in humans.

A tentative UL [equal to the no-observed-adverse-effect level (NOAEL) divided by the uncertainty factor] has been proposed as an appropriate method to establish an NOAEL for humans without using direct clinical studies (3). For L-Trp, a new approach for setting the UL has been proposed. It is based on the observation that the urinary excretion of a nutrient, including...
l-Trp, or its metabolites sharply increases when the dietary intake of the nutrient exceeds the normal range of metabolizing capacity for that nutrient. In other words, the breakpoint of urinary excretion of the nutrient metabolite(s) indicates a metabolic upper intake level (MUL) and dietary intakes leading to urinary concentrations that exceed the metabolic capacity might lead to toxicity.

In the present workshop, we proposed a surrogate index applicable to predicting a UL of l-Trp in rats using a breakpoint in urinary excretion of l-Trp catabolites (4). In a rat experiment, we determined a tentative NOAEL of ≥2000 mg/kg body weight and the lowest-observed-adverse-effect level of ≤5000 mg/kg body weight and we showed that the urinary excretory ratio of anthranilic acid (AnA):kynurenic acid (KA) is the most appropriate surrogate breakpoint index to predict the UL in rats (4).

We have described the fates of l-Trp and Nam in humans (5) and compared them with those of rodents. Consequently, we postulated that using the breakpoint as a surrogate index will also contribute to the prediction of the UL and adverse effects of l-Trp in humans (4). The research upon which this postulation is based is summarized in the sections below. A human experiment to confirm the above hypothesis is ongoing.

Normal Amounts of the Intermediates Involved in the l-Trp–Nam Pathway in the Liver and Blood of Rats

Male rats of the Wistar strain (6 wk old) consumed ad libitum a 20% casein diet for 21 d (6). Whereas trace amounts of quinolinic acid (QA) were found in the liver, the metabolites of the l-Trp–Nam pathway such as kynurenine, 3-hydroxyanthranilic acid (3-HA), xanthurenic acid (XA), AnA, and KA were not detected. The total Nam concentration, which is the sum of the free form of Nam, NAD, and NADP, was ~1.5 μmol/g wet weight. A trace amount of N4-methylnicotinamide (MNA), the catabolite of Nam, was detected in the liver, whereas N3-methyl-2-pyridone-5-carboxamide (2-Py) and N3-methyl-4-pyridone-3-carboxamide (4-Py), both catabolites of MNA, were below the limit of detection. The whole blood kynurenine concentration was 3.5 ± 0.4 μmol/L. 3-HA, XA, AnA, KA, and QA were not detected. The whole blood NAD concentration was 90.6 ± 4.6 μmol/L and that of NADP was 12.1 ± 0.9 μmol/L.

The results indicated that rats immediately eliminated the studied intermediates into urine if the metabolites were not metabolized further along the pathway. MNA was detected at low concentrations in both the liver and blood. NAD and NADP were found in the blood and liver, whereas the other intermediates and Nam catabolites were not detected in the organs. Therefore, the urinary excretion of these compounds reveals a systemic metabolism of l-Trp to Nam.

Urinary Excretory Ratio of AnA:KA as an Index of the UL of l-Trp in Rats

l-Trp is being promoted as a treatment for sleep disorders (7,8) and, consequently, there is the risk of an excessive intake from dietary supplements. It is therefore important to provide a useful index that can monitor excessive l-Trp intake, possibly using changes in metabolite ratios in urine. Our previous studies (9–11) showed the harmful effects of Nam when its intake exceeded the tolerable level. When rats were fed a diet containing very high amounts of Nam that exceeded the tolerable intake level, the urinary excretory ratio of (2-Py + 4-Py):MNA was markedly reduced (9–11). We postulated that an excessive l-Trp intake would also induce a measurable metabolic change that would be reflected in urinary excretion (4). Thus, we investigated l-Trp–Nam metabolism in rats fed a diet containing excessive l-Trp.

Male Wistar rats (3 wk old) consumed ad libitum 1 of 5 diets for 30 d (4): a 20% casein diet as a control or a 20% casein diet supplemented with 0.5, 1.0, 2.0, or 5.0% l-Trp. On d 30, 24-h urine samples were collected.

The food intake and body weight gain of the rats fed the 5% l-Trp diet were lower than those of the other 4 groups. The urinary excretion of such l-Trp catabolites as KA, XA, and 3-HA increased as the intake of l-Trp increased, but the sum of the metabolites MNA, 2-Py, and 4-Py was the same in the 1, 2, and 5% l-Trp diet groups.

The excretion of QA increased gradually with increasing l-Trp intake up to 2% l-Trp in the diet but leveled off in the range of 2–5% l-Trp in the diet. QA is formed from α-amino-β-carboxymuconate-ε-semialdehyde (ACMS), which is formed from 3-HA. The reaction from 3-HA to ACMS is catalyzed by 3-HA oxygenase, an enzyme with an extremely high activity level (715 ± 20 μmol·h⁻¹·g liver⁻¹) compared with those of the other enzymes involved in the l-Trp–Nam pathway (12). In this study, we could not measure ACMS, because the compound is very unstable; however, we speculate that ACMS formation must have increased, because 3-HA was elevated by an excessive l-Trp intake. This means that the activity of the enzyme ACMS decarboxylase (ACMSD) that metabolizes ACMS to α-amino-muconate-ε-semialdehyde (AMS) was also enhanced. The l-Trp–ACMS pathway branches at the stage of this metabolite; one branch is the reaction from ACMS to AMS catalyzed by ACMSD, and the other is the reaction from ACMS to QA due to spontaneous autocyclization (12). The activity of ACMSD would be expected to be induced by l-Trp, because it has been reported that ACMSD activity is altered by various nutritional factors and chemicals in vivo (6,13–26).

The sum of the metabolites MNA, 2-Py, and 4-Py was almost the same in the 1, 2, and 5% l-Trp diet groups, indicating that the metabolism of QA to nicotinic acid mononucleotide was saturated in the 2% l-Trp diet group or the metabolism of ACMS to AMS was accelerated as already mentioned. Quinoline phosphoribosyltransferase (QPRT) metabolizes QA to nicotinic acid mononucleotide. Rao et al. (27,28) reported that QPRT is the rate-limiting enzyme on the l-Trp–Nam pathway in rats. Therefore, QPRT was rate limiting with the 2% l-Trp diet and the enzymatic conversion of QA to nicotinic acid mononucleotide can fully explain the observed metabolic changes.

Interestingly, the urinary excretion of AnA in rats fed the 5% l-Trp diet sharply increased beyond that in rats fed the 2% l-Trp diet. Although KA, 3-HA, and XA increased with increasing l-Trp intake, we cannot clearly explain why the excretion of AnA increased more steeply than the excretions of other metabolites. However, a steep increase in the urinary excretion of AnA was obtained even before any adverse effects were measured. Therefore, we propose that AnA excretion might be used as a predictive biomarker.

Although an excess l-Trp intake could be predicted by measuring the daily excretion of AnA, this method would not be convenient, because 24-h urine samples would be necessary. However, if the value of AnA relative to some l-Trp metabolites could be utilized, an excessive intake of l-Trp could be predicted by a spot urine measurement instead of a 24-h urine sample.

Indeed, we calculated some ratios of AnA: selected l-Trp metabolites. The urinary excretory ratios of AnA:KA, AnA:XA,
and AnA:3-HA for the 5% L-Trp diet group were 83, 20, and 16 times higher, respectively, than the ratios in the control group. These ratios for the 2% L-Trp diet group were 5.5, 3.4, and 3.6 times higher, respectively, than the ratios in the control group. However, the excretion ratios were similar for the 0.5% L-Trp diet, the 1% L-Trp diet, and the control groups. These results show that the urinary excretory ratio of AnA:KA is potentially the most sensitive and appropriate surrogate breakpoint index to predict the UL of L-Trp in rats.

**Urinary Excretion of the Metabolites of the L-Trp-Nam Pathway in Humans**

Ten female Japanese college students aged 21–23 y were housed in the same facility for 9 d and consumed a semipurified diet for 7 d (5). The total energy intake was ~7500 kJ/d, total protein intake was ~55 g/d, and the total fat:energy ratio was 25%. The diet contained 674 mg/d L-Trp (~3.3 mmol/d) and no niacin.

**Effects of Long-Term Administration of a Multivitamin Preparation on Nam Catabolism in Young Japanese Men**

Because Nam is biosynthesized from L-Trp in human liver, L-Trp toxicity might be related to excess formation of Nam. The toxicity of a large amount of Nam has been reported (29–32) and the UL has been set at 300 mg/d (3).

Twenty-one healthy, free-living men aged 21–24 y were divided into 3 groups (33). One group was administered a placebo, the second group received 150 mg/d of Nam as part of a multivitamin preparation for 44 wk [the vitamin composition is shown in (33) and the content of Nam was 37.5 mg/tablet]. The urinary excretion of Nam itself was not detected even when 150 mg of Nam was consumed. The urinary excretions of MNA, 2-Py, and 4-Py in the placebo control group were ~30, 40, and 4 μmol/d, respectively. The urinary excretions of MNA, 2-Py, and 4-Py each increased ~5- and 10-fold after administration of 75 and 150 mg of Nam, respectively, compared with those of the nonadministration period. Based on these results, men can fully metabolize the intake of up to at least 150 mg/d of Nam. If the conversion ratio of L-Trp:Nam is 1:60 on a weight basis, 150 mg of Nam is equivalent to 9 g of L-Trp.

**Development of an Index of the MUL Instead of the UL of L-Trp for Humans**

L-Trp is one of the limiting amino acids in many proteins. The administration of the free form of L-Trp to improve nutritional status is superior to the administration of the bound form of L-Trp, such as in proteins and large peptides. The free form of L-Trp is also a good precursor of Nam compared with the bound form of L-Trp (34,35). Furthermore, L-Trp has shown some effectiveness for treatment of a variety of other conditions typically associated with low serotonin concentrations in the brain. The UL of L-Trp for humans is not well known and this amount cannot be determined with human participants due to ethical considerations. The aim of the present workshop was to identify biomarkers that could be used before the appearance of adverse effects of L-Trp. We propose the development of an index of the MUL for L-Trp to be used instead of the UL; these results show that the urinary excretory ratio of AnA:KA for rats is potentially the most sensitive and appropriate surrogate breakpoint index to predict the UL of L-Trp.

A human experiment for determining the metabolic breakpoint as a surrogate index for L-Trp safety is currently ongoing.

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**Literature Cited**


