

Tryptophan Catabolism and Vitamin B-6 Status Are Affected by Gender and Lifestyle Factors in Healthy Young Adults^{1–3}

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Abstract

Background: Abnormalities of tryptophan (Trp) metabolism through the kynurenine (Kyn) pathway have been reported in various diseases; however, nutritional and lifestyle factors that affect this pathway in healthy individuals are not well documented.

Objective: Our aim was to examine the effect of vitamin B-6 status and lifestyle factors including the use of vitamin B-6 supplements, alcohol, smoking, and oral contraceptives on Trp and its Kyn metabolites in a cohort of 2436 healthy young adults aged 18–28 y.

Methods: Anthropometric and lifestyle data were collected by questionnaire. Participants provided blood samples for analysis of Trp, Kyn, anthranilic acid, kynurenic acid (KA), 3-hydroxykynurenine (HK), 3-hydroxyanthranilic acid (HAA), and xanthurenic acid (XA). Vitamin B-6 species were also measured.

Results: Serum Trp metabolites were 10–15% higher among men ($n = 993$) compared with women ($n = 1443$; $P < 0.0001$), except for HK and XA. In all participants, serum Trp was positively associated with plasma pyridoxal 5'-phosphate (PLP; $r = 0.28$, $P < 0.0001$), reaching a plateau at PLP concentrations of ~ 83 nmol/L. HK was inversely associated with PLP ($r = -0.14$, $P < 0.01$). Users of vitamin B-6 supplements ($n = 671$) had 6% lower concentrations of HK than nonusers ($n = 1765$; $P = 0.0006$). Oral contraceptive users ($n = 385$) had lower concentrations of KA (20.7%) but higher XA (24.1%) and HAA (9.0%) than did nonusers ($n = 1058$; $P < 0.0001$). After adjustment for gender and other lifestyle variables, XA concentrations were 16% higher in heavy drinkers ($n = 713$) than in never or occasional drinkers ($n = 975$; $P = 0.0007$). Concentrations of 2 other essential amino acids, methionine and arginine, also were positively associated with serum Trp ($r = 0.65$ and 0.33 , respectively; $P < 0.0001$).

Conclusions: In this population of healthy young adults, gender has the largest influence on serum Kyn metabolite concentrations. The significant covariance of Trp with unrelated amino acids suggests that protein intake may be an important consideration in evaluating Kyn metabolism. *J Nutr* 2015;145:701–7.

Keywords: tryptophan, kynurenine, 3-hydroxykynurenine, pyridoxal 5'-phosphate, vitamin B-6, protein

Introduction

Tryptophan (Trp) catabolism through kynurenine (Kyn)¹³ has attracted considerable research interest because altered metabolite

concentrations down this pathway have been reported in diseases such as HIV infection, Alzheimer disease, cancer, and diabetes (1–4). The Kyn pathway is the major route of Trp catabolism (4), with indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO) enzymes catalyzing the first rate-limiting step of Trp catabolism in a tissue-dependent manner (5). IDO is involved in

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³ Supplemental Figure 1 and Supplemental Tables 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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¹³ Abbreviations used: AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; IDO, indoleamine 2,3-dioxygenase; KA, kynurenic acid; KAT, kynurenine aminotransferase; KTR, kynurenine to tryptophan ratio; Kyn, kynurenine, KYNU, kynureninase; PA, 4-pyridoxic acid; PL, pyridoxal; PLP, pyridoxal 5'-phosphate; TDO, tryptophan 2,3-dioxygenase; XA, xanthurenic acid.

extrahepatic Trp catabolism, and its activity is induced by proinflammatory modulators such as IFN- γ , TNF- α , IL-1, IL-2, and LPS (6, 7). TDO is a constitutive hepatic enzyme, which is inducible by stress hormones such as glucagon and regulated by the availability of its substrate, L-Trp (5).

Two key enzymes in the Kyn pathway, kynurenine aminotransferase (KAT) and kynureninase (KYNU), are dependent on pyridoxal 5'-phosphate (PLP) as a cofactor. KAT catalyzes both the transamination of Kyn to kynurenic acid (KA) and 3-hydroxykynurenine (HK) to xanthurenic acid (XA). KYNU catalyzes the conversion of Kyn into anthranilic acid (AA) and HK into 3-hydroxyanthranilic acid (HAA). Human KYNU is reported to have a 20-fold higher affinity for HK than for Kyn (8), favoring the production of HAA over AA. There is evidence that vitamin B-6 deficiency in humans impairs Trp metabolism (4, 9–13).

Studies have reported altered concentrations of Kyn metabolites in the serum of patients with disease conditions, including diabetes (4, 14, 15), inflammatory bowel disease (16), and neurodegenerative disorders such as Huntington, Alzheimer, and Parkinson disease (17) and with brain tumor pathogenesis (18). Nevertheless a causal relation has yet to be demonstrated in any of these conditions.

The link between vitamin B-6, Trp, and Kyn metabolites has been investigated in only one large study of apparently healthy individuals (19). That study reported only on middle-aged and elderly subjects. We examined the serum concentrations of Kyn metabolites in a large healthy cohort of young adults ($n = 2436$; median age: 22 y) to establish baseline ranges and to explore the effect of lifestyle factors, including oral contraceptive use, alcohol intake, vitamin B-6 supplement use, and smoking.

Methods

Subjects. The Trinity Student Study enrolled students attending the University of Dublin, Trinity College, between February 2003 and February 2004. Eligibility criteria included age between 18 and 28 y, no current serious medical condition, and Irish ethnicity based on origins of grandparents. A total of 2524 subjects were eligible to participate. Ethical approval was obtained from the Dublin Federated Hospitals Research Ethics Committee, which is affiliated with University of Dublin, Trinity College, and subjects gave written informed consent. The study was reviewed by the Office of Human Subjects Research at the NIH. Further details were published elsewhere (20–22). Fifteen subjects with no questionnaire data and one duplicate sample were excluded, leaving 2508 valid participants whose samples were assigned for analysis. Data were available for Trp, Kyn, and most Kyn pathway metabolites for 2436 subjects, forming the data set for the current study. Blood samples and questionnaire data were coded and made anonymous before analysis.

Blood collection and biochemical analyses. Nonfasting blood samples were collected on the day of the interview. Samples were kept cool, then separated within 3 h of collection and stored at -80°C until analysis. Vitamin B-6 species [PLP, pyridoxal (PL), and 4-pyridoxic acid (PA)] were measured in plasma, and Trp metabolites (AA, HAA, HK, KA, XA, and Kyn), cotinine, and selected amino acids (Arg, Met) were measured in serum by using high-throughput LC–tandem MS assays (23) in the laboratory of Bevitall AS (www.bevital.no). Complete blood cell counts were measured by using a Sysmex F-800 cell counter calibrated with CBC-ST Plus hematology controls (low, normal, and high) (R&D Systems). Liver function (γ -glutamyltransferase, alanine aminotransferase, aspartate aminotransferase, and total bilirubin) and kidney function (creatinine, uric acid, and urea) tests were performed by using an Abbott architect (Clayton Laboratories). Inter- and intra-assay CVs were $<4.8\%$.

Questionnaire data. Information on physiologic factors such as age, gender, height, weight, and medical conditions was collected together

with data on lifestyle habits such as diet, smoking, oral contraceptive use, and consumption of alcohol, fortified foods, and supplements.

Supplement intake. Participants reported their supplement use in the past week and over an average month. Reported quantity and frequency of supplement intake were used to calculate each individual's supplemental nutrient intake over an average month. Nutrient information was obtained for each supplement from labels and manufacturers' information. Amounts of nutrients listed were converted to micrograms of nutrient per portion (tablet or liquid) according to standard conversion methods.

Smoking status. Smoking status was assessed in 2 ways, by questionnaire (available for all participants) and by serum cotinine concentration (available for 2105 participants). Questionnaires were used to divide smoking status into 3 groups: 1 = nonsmokers, 2 = moderate smokers (smoking cigars or a pipe occasionally or 1–19 cigarettes/d or a 25-g pack of tobacco in >1 wk), and 3 = heavy smokers (smoking >20 cigarettes/d or a 25-g pack of tobacco in <1 wk or regular use of cigars or a pipe). Cotinine data were divided into 3 categories for analysis as described previously (19, 24): ≤ 85 nmol/L, between 86 and 1199 nmol/L, and ≥ 1200 nmol/L.

Alcohol intake. Participants reported average alcohol consumption by using a quantity-frequency-beverage-specific questionnaire. Intake, converted to grams of ethanol per day, was categorized into never/occasional (0 to <15 g/d), light to moderate (15 to <30 g/d), and heavy (≥ 30 g/d).

Statistical analysis. Most metabolites had non-normal distributions, and data in tables are expressed as medians (5th–95th percentile ranges). Statistical analyses of data were performed by using SAS version 9.3. Independent Student's t tests were used to determine significant ($P < 0.05$) differences between groups in Table 1. Chi-square tests were used to compare differences in categorical data. For association analysis, metabolite partial correlation scatter plots were constructed on log-transformed data and Pearson correlation coefficients were reported. General linear models were used to fit serum concentrations of the 7 Trp metabolites as well as plasma PLP, PL, and PA by using the ANOVA method of least squares. Four factors were included in the model as independent variables: grams of alcohol per day, smoking category, use of vitamin B-6-containing supplements, and a dummy variable to address the confounding effect of gender and oral contraceptive use (0 = male, 1 = female nonusers, and 2 = female users). Rank normal inverse transformations were applied to the 10 metabolites to satisfy the normality assumption. For each metabolite, type III sum of squares and F distribution P values were calculated for alcohol, smoking, supplement use, and the combined factor that treated gender and oral contraceptive use. To further evaluate the effect of gender and contraceptive usage by women, contrast analyses were performed on the basis of linear combinations of the average of corresponding levels of the gender–oral contraceptive variable. To explore nonlinear relations between metabolites, generalized additive models were constructed by using R statistical software, version R 3.1.1 (25).

Results

Characteristics of the study population

General characteristics of participants in the Trinity Student Study population are shown in Table 1; 59.2% of the subjects were women. Liver and kidney function tests and hematology variables were all within clinically normal reference ranges (26) (data not shown).

Trp metabolites and vitamin B-6 species blood concentrations

Data for serum Trp metabolite and vitamin B-6 species concentrations are presented in Table 2. Men had ~ 10 –15% higher concentrations of all measured Trp metabolites except for HK,

TABLE 1 General and lifestyle characteristics of healthy young adults from the Trinity Student Study cohort¹

Variable	Total (n = 2436)	Women (n = 1443)	Men (n = 993)
Age, ² y	22.0 (20.0–26.0)	22.0 (20.0–25.0)	23.0 (20.0–26.0)***
BMI, ² kg/m ²	22.5 (18.9–28.5)	22.2 (18.7–28.4)	23.0 (19.2–28.8)***
Smoking in past 4 mo, ³ n (%)	753 (30.9)	436 (30.2)	317 (31.9)
Alcohol, ³ n (%)			
Never/occasional	975 (40.0)	744 (51.5)	231 (23.2)***
Moderate	745 (30.6)	443 (30.7)	302 (30.4)
Heavy	713 (29.2)	255 (17.7)	458 (46.1)***
Vitamin B-6 supplement use in past mo, ³ n (%)	671 (27.5)	445 (30.8)	226 (22.7)***
Vegetarian diet, ³ n (%)	104 (4.3)	70 (4.9)	37 (3.7)

¹ Values are medians (5th–95th percentiles) for continuous variables. ***Different from women, $P < 0.0001$.

² Student's independent t test was used to test differences between log-transformed continuous variables.

³ Chi-square tests were used to test differences between categorical variables.

with KA being 33% higher than in women. Significant positive correlations in the range of 0.13–0.67 were observed between Trp and its metabolites (**Supplemental Table 1**); the strongest correlations ($r > 0.53$) were found between HK and its PLP-dependent products XA and HAA. Trp also showed significant positive correlations with the amino acids Met ($r = 0.65$) and Arg ($r = 0.33$). PLP also correlated positively with all Trp metabolites ($r = 0.10$ – 0.28) except for HK, which had an inverse relation ($r = -0.14$, $P < 0.0001$).

To show nonlinear trends, generalized additive model plots were constructed in which concentrations of PLP (**Figure 1A, B**) and Trp (**Figure 1C, D**) were plotted against Kyn pathway metabolites. Approximately linear positive relations were observed between Trp and most Kyn pathway metabolites. For PLP, the positive relation with Trp metabolites leveled off above the median PLP concentration for this cohort (83 nmol/L; **Supplemental Figure 1**). Similar relations were observed with other metabolites (data not shown). In contrast, the positive relation between HK and Trp was largely confined to very high blood Trp concentrations, and the negative trend between HK and PLP was largely confined to low PLP concentrations.

Effect of lifestyle factors

Vitamin B-6 supplement use. Approximately 27% of subjects reported recent use of supplements containing vitamin B-6; 66%

of the users were women (**Table 1**). After adjusting for gender, contraceptive use, smoking, and alcohol intake, those consuming vitamin B-6 supplements had significantly lower concentrations of HK than did nonusers (-5.2% ; $P = 0.0006$) (**Table 3**). No other Trp metabolite changed with vitamin B-6 supplement use. All 3 vitamin B-6 species were considerably higher in supplement users ($P < 0.0001$).

Oral contraceptive use. In this study, 26.7% of women used oral contraceptives (**Table 4**). Even after adjustment for supplemental vitamin B-6 use, smoking, and alcohol intake, XA and HAA concentrations were significantly higher in contraceptive users vs. nonusers (XA: $+31.8\%$; HAA: $+9.9\%$; $P < 0.0001$), whereas Kyn and KA were lower (Kyn: -3.7% ; $P = 0.005$; KA: -20.7% ; $P < 0.0001$). HK itself, the putative marker of vitamin B-6 deficiency, was not affected by oral contraceptive use ($P = 0.10$). Moreover, although PLP was lower among contraceptive users ($P = 0.013$), there was no difference in concentrations of the other measured B-6 vitamins (PL: $P = 0.19$; PA: $P = 0.28$).

Smoking and alcohol. Smoking was associated with considerably lower PLP status (-16.2% in nonsmokers vs. heavy smokers; $P = 0.0007$) on the basis of self-reported questionnaire data (**Supplemental Table 2**). PL and PA concentrations showed no relation with smoking ($P = 0.60$ and $P = 0.74$, respectively).

TABLE 2 Effects of gender on serum concentrations of tryptophan and kynurenine pathway metabolites and vitamin B-6 species in healthy young adults from the Trinity Student Study cohort¹

Variable	Total (n = 2436)	Women (n = 1443)	Men (n = 993)
Tryptophan, $\mu\text{mol/L}$	70.0 (51.2–94.1)	67.3 (49.3–89.1)	73.4 (55.2–96.7)***
Kynurenine, $\mu\text{mol/L}$	1.3 (0.9–1.9)	1.3 (0.9–1.7)	1.4 (1.0–2.0)***
Kynurenine:tryptophan ratio, nmol/L: $\mu\text{mol/L}$	19.8 (14.3–27.6)	19.3 (14.1–27.1)	20.3 (14.8–27.9)***
3-Hydroxykynurenine, ² nmol/L	29.9 (17.7–49.8)	29.8 (17.5–50.7)	30.1 (18.9–48.5)
Kynurenic acid, nmol/L	46.1 (23.0–84.1)	40.4 (20.9–74.4)	53.9 (29.6–92.5)***
Xanthurenic acid, nmol/L	17.3 (7.0–40.4)	16.5 (6.3–41.8)	18.6 (8.01–38.8)
Anthranilic acid, nmol/L	13.8 (8.3–24.4)	13.4 (7.7–23.0)	14.6 (9.00–25.2)***
3-Hydroxyanthranilic acid, ³ nmol/L	40.8 (21.7–79.9)	39.6 (20.2–76.5)	42.9 (24.1–81.9)**
Pyridoxal 5'-phosphate, nmol/L	82.7 (35.6–231)	74.7 (32.8–241)	92.4 (43.8–226)***
Pyridoxal, nmol/L	18.9 (9.4–55.3)	17.6 (8.9–64.9)	20.3 (10.7–50.5)
Pyridoxic acid, nmol/L	32.3 (13.7–129)	29.9 (12.5–140)	35.7 (16.9–115)

¹ Values are medians (5th–95th percentiles). ****Different from women: ** $P = 0.007$, *** $P < 0.0001$. Differences were tested on rank normal-transformed data by using general linear models adjusted for use of vitamin B-6 supplements, oral contraceptive use, and smoking and alcohol status.

² Values were available for 1346 female and 918 male participants.

³ Values were available for 1343 female and 916 male participants.

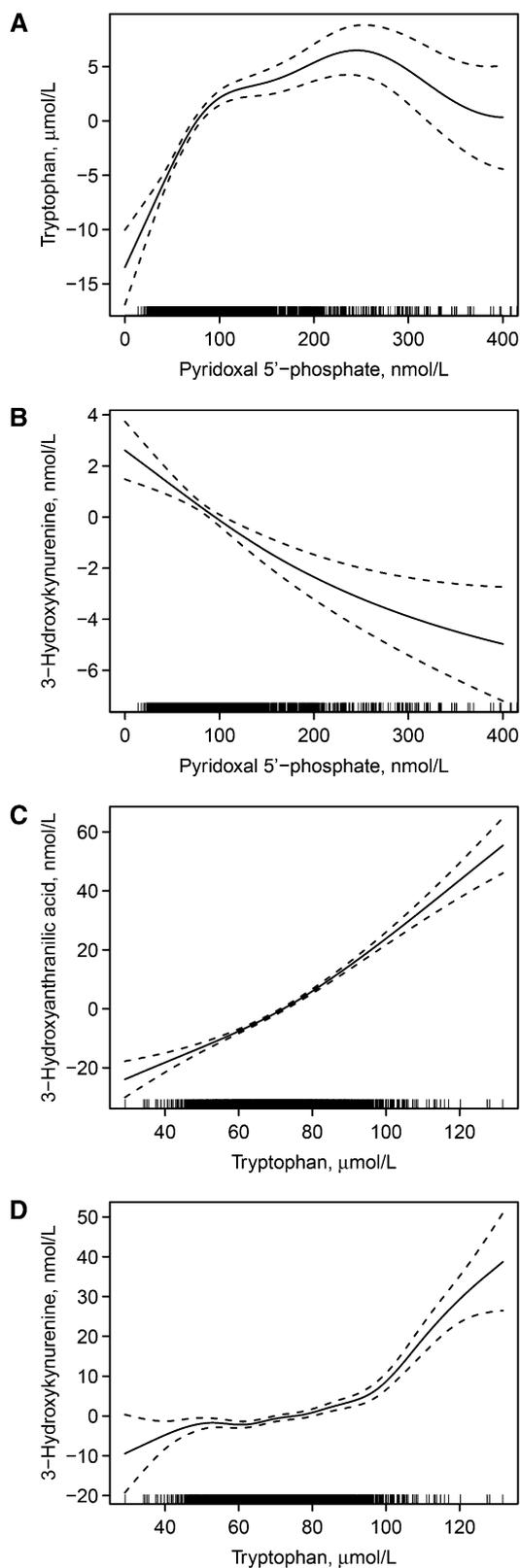


FIGURE 1 Generalized additive model association plots between tryptophan, pyridoxal 5-phosphate, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid in combined male and female healthy young adults of the Trinity Student Study cohort. Associations between tryptophan, pyridoxal 5-phosphate, and the kynurenine pathway metabolites 3-hydroxykynurenine and 3-hydroxyanthranilic acid are shown. The area between the dotted lines shows the 95% CI of the fitted model. The x axis shows the concentrations of x variables, with black bars representing sample density. The y axis shows the

Smoking had no substantial effect on any measured Trp pathway metabolites. Similar results were obtained from analysis of cotinine data (data not shown).

Overall, 29.2% (713) of subjects were classified as heavy drinkers (**Supplemental Table 3**). A substantially higher proportion of men (46.4%) than women (17.5%) exceeded the recommended maximum intake of 32 and 24 grams of alcohol/d, respectively. After adjusting for gender, contraceptive use in women, supplemental vitamin B-6 use, and smoking, high alcohol intake was associated with increased XA concentrations ($P = 0.0007$). Vitamin B-6 species were not changed by alcohol intake after adjustment.

The Kyn to Trp ratio (KTR), expressed as nmol/L Kyn to $\mu\text{mol/L}$ Trp, was evaluated as a measure of increased flux through the Trp oxidation pathway (12, 19). No additional discrimination of effect was observed by using this variable.

Discussion

This study is the first to explore the effects of lifestyle habits on serum concentrations of Kyn pathway metabolites in a large homogeneous population of healthy young adults. Our results provide useful normal-range data for the study of Trp catabolism through the Kyn pathway, demonstrating clear gender differences. Trp concentrations were strongly correlated with most of its downstream Kyn metabolites. Moreover, serum concentrations of other essential amino acids (Met and Arg), not related to this pathway, were correlated both with Trp metabolites and with PLP, suggesting that protein intake may be an important factor not previously explored in relation to Kyn metabolism. These observations contrast with a previous study, which reported a decrease in PLP status with protein intake (27). The negative association between PLP concentrations and both smoking and oral contraceptive use seen in this cohort of healthy subjects parallels that seen in inflammatory conditions (9, 28–31) or pregnancy (32, 33), but the associations of these lifestyle factors on Trp pathway metabolites do not mirror the pattern seen in simple PLP deficiency and suggest, alternatively, a redistribution of flux through this pathway.

Our data confirm earlier reports that HK is the only Kyn metabolite that increases as vitamin B-6 concentration decreases (29, 30). HK in our population was also affected by vitamin B-6 supplement use, raising the possibility that KAT and KYNU enzymes, at the HK metabolic branch point, might be sensitive to PLP status well into the normal PLP range.

The median concentrations of Trp metabolites in this study are comparable with concentrations reported by other studies in clinical cohorts and older subjects (12, 16, 19, 23, 31, 34). In a cohort with suspected coronary artery disease, slightly higher concentrations of Kyn and lower concentrations of PLP were reported (12). This could be due to the fact that Kyn increases significantly with age (19) and that PLP concentrations are decreased and Kyn is increased in states of inflammation (35–37). Moreover, this difference could also be due to higher vitamin B-6 concentrations in our study cohort as a result of supplement use, fortified food intake, and/or alcohol consumption (38).

distribution of y variables relative to the median value (represented as 0). Panel A includes 2436 observations, panels B and D include 2264 observations, and panel C includes 2259 observations.

TABLE 3 Effects of vitamin B-6 supplement use on tryptophan and kynurenine pathway metabolites in healthy young adults from the Trinity Student Study cohort¹

Variable	Total (n = 2436)	Vitamin B-6 supplement users (n = 671)	Non-supplement users (n = 1765)
Tryptophan, μmol/L	70.0 (51.2–94.1)	70.3 (51.1–95.3)	69.8 (51.3–93.7)
Kynurenine, μmol/L	1.3 (0.9–1.9)	1.3 (0.9–1.9)	1.3 (0.9–1.9)
Kynurenine:tryptophan ratio, nmol/L:μmol/L	19.8 (14.3–27.6)	19.3 (14.0–27.3)	19.9 (14.4–27.8)
3-Hydroxykynurenine, ² nmol/L	29.9 (17.7–49.8)	28.8 (17.4–48.2)	30.4 (17.9–50.3)**
Kynurenic acid, nmol/L	46.1 (23.0–84.1)	44.4 (21.7–81.5)	46.5 (23.5–85.9)
Xanthurenic acid, nmol/L	17.3 (7.0–40.4)	16.7 (6.8–41.6)	17.6 (7.0–39.9)
Anthranilic acid, nmol/L	13.8 (8.3–24.4)	13.8 (8.6–24.6)	13.9 (8.3–24.3)
3-Hydroxyanthranilic acid, ³ nmol/L	40.8 (21.7–79.9)	40.5 (21.1–83.1)	40.8 (22.3–78.1)
Pyridoxal 5'-phosphate, nmol/L	82.7 (35.6–231)	106.2 (42.8–368)	76.8 (34.3–179)***
Pyridoxal, nmol/L	18.9 (9.4–55.3)	23.9 (11.0–146)	17.6 (9.1–40.7)***
Pyridoxic acid, nmol/L	32.3 (13.7–129)	43.7 (16.7–290)	29.9 (13.3–94.0)***

¹ Values are medians (5th–95th percentiles). ****Different from non-supplement users: ***P* = 0.0006, ****P* < 0.0001. Differences were tested on rank normal-transformed data by using general linear models adjusted for gender, oral contraceptive use, and smoking and alcohol status.

² Values were available for 622 vitamin B-6 supplement users and 1642 nonusers.

³ Values were available for 622 vitamin B-6 supplement users and 1637 nonusers.

In agreement with earlier work (39), we found lower concentrations of PLP, with no change in PL or PA concentrations, in women taking oral contraceptives, possibly reflecting a redistribution of PLP at the tissue level rather than vitamin B-6 deficiency, as suggested by others (40, 41). The changes in Trp metabolites among contraceptive users compared with women not using contraceptives suggest that the pathway is under substantial estrogenic influence. Mason et al. (42) reported that estrogenic steroids reduce the affinity of KYNU and KAT for PLP. Other studies found that estrogenic steroids increased hepatic TDO activity (32, 43). Hormonal status through the menstrual cycle could therefore be an important factor in Trp degradation in women of reproductive age (44).

Of the 3 vitamin B-6 species, only PLP decreased significantly with smoking after adjusting for all other measured factors. This effect was described in previous studies (31, 45–47). We did not see any appreciable change in Kyn metabolites among smokers. In contrast, high alcohol intake was associated with a significant increase in XA after adjustment for other

factors, although further research is needed to understand the mechanism. The potential effect of antioxidant activity on Trp metabolism is worth noting here. Antioxidants and antioxidative preservatives commonly added to nutritional products or drinks have been associated with increased IDO activity (in vitro), which can result in increased Trp degradation (48, 49). Given the increasing use of antioxidants to promote health and the widespread use of alcohol, lifestyle and nutrition choices will become an increasingly important influence on Trp metabolism.

Numerous studies have shown that Trp degradation increases with inflammation and KTR is considered a sensitive marker of this relation (1, 12, 19, 30, 31, 50). The participants in this cohort were healthy young individuals; thus, the KTR values reported here are lower than those reported in cohorts with inflammation (50). Men had higher KTRs than did women, showing the effect of gender on Trp catabolism (51), but otherwise no substantial effects of lifestyle variables on KTR were noted in this cohort.

TABLE 4 Effects of oral contraceptive use on tryptophan and kynurenine pathway metabolites in healthy young women from the Trinity Student Study cohort¹

Variable	Total (n = 1443)	Oral contraceptive users (n = 385)	Nonusers (n = 1058)
Tryptophan, μmol/L	70.0 (51.2–94.1)	67.3 (50.1–88.2)	67.3 (49.1–90.0)
Kynurenine, μmol/L	1.3 (0.9–1.9)	1.2 (0.9–1.7)	1.3 (0.9–1.7)**
Kynurenine:tryptophan ratio, nmol/L:μmol/L	19.8 (14.3–27.6)	18.8 (14.1–26.6)	19.6 (14.1–27.3)**
3-Hydroxykynurenine, ² nmol/L	29.9 (17.7–49.8)	30.3 (17.5–51.4)	29.6 (17.4–50.1)
Kynurenic acid, nmol/L	46.1 (23.0–84.1)	33.9 (19.8–69.4)	42.8 (22.0–76.2)***
Xanthurenic acid, nmol/L	17.3 (7.0–40.4)	20.3 (7.3–48.8)	15.4 (6.2–39.6)***
Anthranilic acid, nmol/L	13.8 (8.3–24.4)	12.9 (7.4–21.5)	13.6 (8.0–24.1)
3-Hydroxyanthranilic acid, ³ nmol/L	40.8 (21.7–79.9)	42.1 (22.8–80.5)	38.3 (19.7–73.9)***
Pyridoxal 5'-phosphate, nmol/L	82.7 (35.6–231)	68.7 (29.6–208)	76.6 (33.6–247)*
Pyridoxal, nmol/L	18.9 (9.4–55.3)	17.5 (8.8–76.4)	17.8 (9.0–64.5)
Pyridoxic acid, nmol/L	32.3 (13.7–129)	29.9 (13.5–138)	29.9 (12.3–142)

¹ Values are medians (5th–95th percentiles). ****Different from oral contraceptive users: **P* = 0.013, ***P* = 0.005, ****P* < 0.0001. Differences were tested on rank normal-transformed data by using general linear models adjusted for use of vitamin B-6 supplements and smoking and alcohol status.

² Values were available for 367 female oral contraceptive users and 979 nonusers.

³ Values were available for 366 female oral contraceptive users and 977 nonusers.

A strength of this study is the sample size ($n = 2436$). To our knowledge, this is the largest study to date conducted in a cohort of healthy young adults and substantially adds to current information on normal-range data of Trp metabolites and how they are affected by different lifestyle factors (19). Limitations include the lack of detailed protein intake data and the fact that lifestyle data were self-reported, which can lead to under- or overestimation of true effects. Finally, our data on serum concentrations are steady state levels, which may only give a reflection of metabolic flux at the cellular level.

In conclusion, our data confirm that gender, vitamin B-6 supplement use, oral contraceptive use, and alcohol intake have important effects on vitamin B-6-dependent Trp metabolism. HK has the potential to be a sensitive marker that reflects vitamin B-6 status within the cell. Protein intake may be an important factor to consider in an analysis of associations between Trp pathway metabolites and disease. This work should provide a useful baseline for future investigations of Trp metabolism in health and in disease states.

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Supplemental Table 1: Pearson correlations between serum tryptophan metabolites in healthy young adults from the Trinity Student Study cohort ¹

	Trp ²	Kyn	HK	KA	XA	AA	HAA	Met	Arg
Kyn	0.45**								
HK	0.23**	0.45**							
KA	0.28**	0.51**	0.43**						
XA	0.45**	0.28**	0.53**	0.54**					
AA	0.16**	0.33**	0.13**	0.35**	0.19**				
HAA	0.51**	0.38**	0.61**	0.44**	0.67**	0.20**			
Met	0.65**	0.30**	0.27**	0.37**	0.38**	0.15**	0.51**		
Arg	0.33**	0.09**	0.05*	0.12**	0.12**	0.03	0.22**	0.58**	
PLP	0.28**	0.10**	-0.14**	0.16**	0.12**	0.12**	0.13**	0.18**	0.12**

¹Pearson correlation coefficients were calculated on log transformed data

²Abbreviations: AA, Anthranilic acid; Arg, Arginine; HAA, 3-Hydroxyanthranilic acid; HK, 3-Hydroxykynurenine; KA, Kynurenic acid; Kyn, Kynurenine; Met, Methionine; PLP, Pyridoxal 5'-phosphate and Trp, Tryptophan.

**Correlation is significant at P<0.0001; *Correlation is significant at P=0.024

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Supplemental Table 2: Effect of smoking on tryptophan and kynurenine pathway metabolites in healthy young adults from the Trinity Student Study cohort

Variable	Non-active smokers	Moderate smokers ³	Heavy smokers
<i>n</i>	1,617	727	36
Tryptophan $\mu\text{mol/L}$ ¹	70.4 (51.4,93.6)	68.7 (51.1,95.3)	71.5 (49.2,97.0)
Kynurenine $\mu\text{mol/L}$	1.3 (1.0,1.9)	1.4 (0.9,1.9)	1.3 (1.0,2.0)
Kynurenine/Tryptophan ratio $\text{nmol/L}/\mu\text{mol/L}$	19.6 (14.3,27.1)	20.3 (14.4,28.3)	19.6 (13.5,29.6) ^{*2}
3-Hydroxykynurenine nmol/L ³	29.9 (17.8,49.8)	29.7 (17.7, 49.9)	34.1 (18.8,47.1)
Kynurenic acid nmol/L	45.6 (23.0,82.1)	47.1 (23.2,88.6)	44.4 (18.4,85.4)
Xanthurenic acid nmol/L	17.3 (7.24,40.2)	17.1 (6.79,39.7)	16.9 (6.76,38.2)
Anthranilic acid nmol/L	14.0 (8.6,25.1)	13.6 (7.88, 23.3)	13.1 (7.7,26.4)
3-Hydroxyanthranilic acid nmol/L ⁴	40.9 (22.2,81.1)	40.2 (20.3,77.9)	42.1 (20.7,65.6)
Pyridoxal 5'-phosphate nmol/L	84.8 (36.4,233)	79.1 (34.9,223)	53.6 (30.4,162) ^{***}
Pyridoxal nmol/L	19.1 (9.46,54.8)	18.7 (9.38,55.8)	15.9 (9.32,66.3)
Pyridoxic acid nmol/L	32.9 (13.7,130)	31.5 (13.3,139)	26.3 (13.4,101)

¹ Median (5th, 95th percentiles)

² Differences across the three smoking categories were tested on rank normal transformed data using general linear models adjusted for gender, contraceptive use, use of vitamin B-6 supplements and alcohol status.

³ Values were available for 1508 non-active, 667 moderate and 34 heavy smokers.

⁴ Values were available for 1506 non-active, 665 moderate and 33 heavy smokers.

Differences across groups, *P=0.03, *** P<0.0007.

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Supplemental Table 3: Effect of alcohol intake on tryptophan and kynurenine pathway metabolites in healthy young adults from the Trinity Student Study cohort

Variable n	Never/Occasional 0- 14.98 g/day 975	Moderate 15.0-29.9 g/day 745	Heavy 30.0-165 g/day 713
Tryptophan $\mu\text{mol/L}$ ¹	69.4(49.2,92.0)	70.4(51.6,93.9)	70.5(53.7,96.5)
Kynurenine $\mu\text{mol/L}$	1.3(0.9,1.8)	1.3(1.0,1.9)	1.4(1.0,1.9)
Kynurenine/Tryptophan ratio $\text{nmol/L}/\mu\text{mol/L}$	19.5 (14.2,27.2)	19.9 (14.3,27.2)	20.1 (14.5,28.0)
3-Hydroxykynurenine nmol/L ³	29.1(17.4,48.8)	30.4(17.7,49.4)	30.2(18.9,51.5)
Kynurenic acid nmol/L	42.2(21.2,76.7)	46.7(23.6,84.0)	50.3(26.1,89.4)
Xanthurenic acid nmol/L	15.9(6.3,38.4)	18.1(7.05,41.2)	18.4(7.7,42.0) ^{***2}
Anthranilic acid nmol/L	13.8(8.3,25.3)	14.1(8.35,24.0)	13.6(8.2,24.1)
3-Hydroxyanthranilic acid nmol/L ⁴	39.6(19.9,77.6)	41.3(22.3,81.1)	42.4(24.5,81.2)
Pyridoxal 5'-phosphate nmol/L	79.6(35.2,236)	81.1(35.4,225)	88.9(38.2,239)
Pyridoxal nmol/L	18.2(8.9,53.1)	18.7(9.79,57.8)	20.3(9.9,56.3)
Pyridoxic acid nmol/L	31.6(12.9,134)	32.7(14.8,131)	33.0(14.0,115)

¹ Median (5th, 95th percentiles)

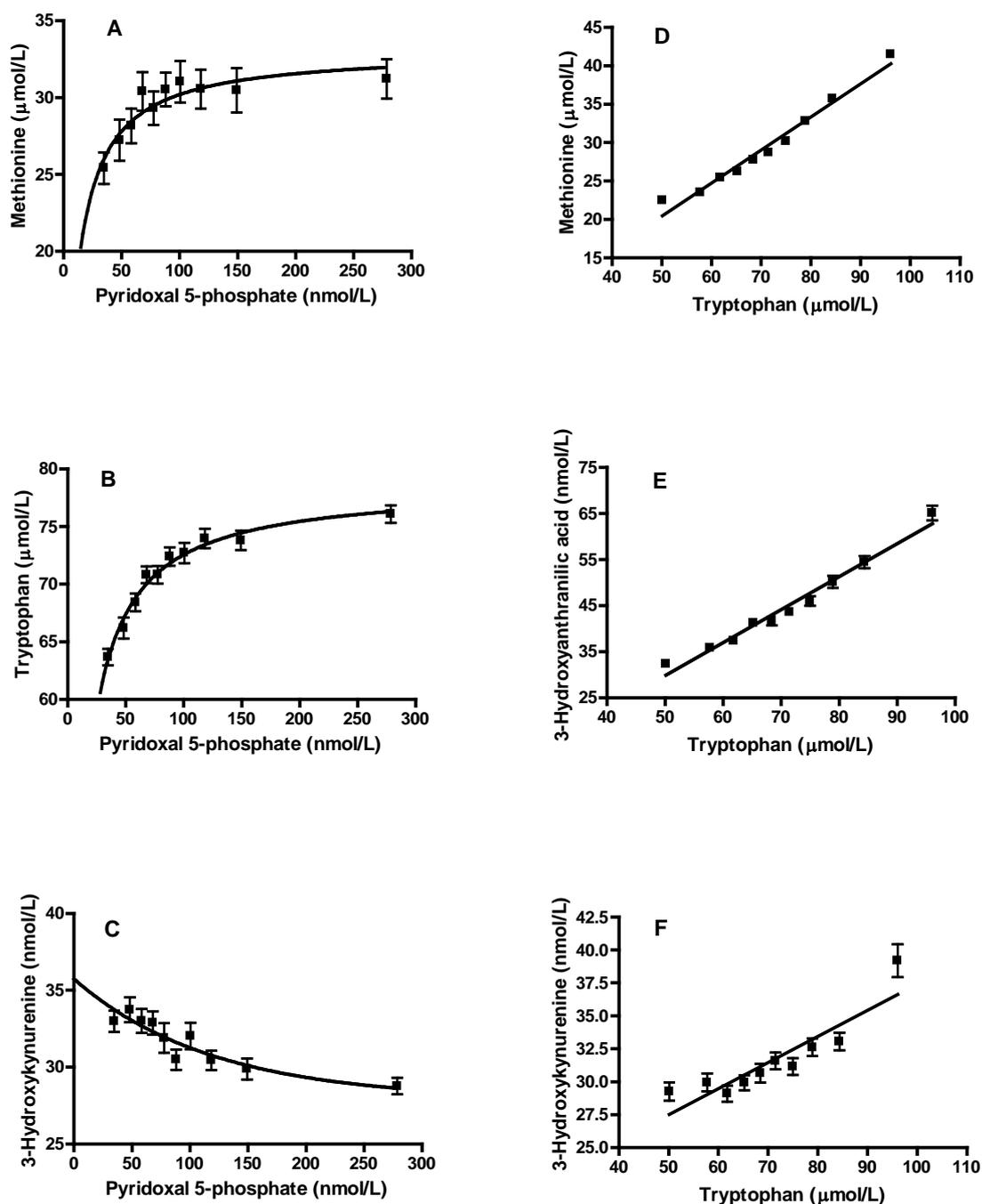
² Differences across the three alcohol categories were tested on rank normal transformed data using general linear models adjusted for gender, contraceptive use, use of vitamin B-6 supplements and smoking status

³ Values were available for 904 never/occasional, 700 moderate and 658 heavy alcohol intake participants

⁴ Values were available for 901 never/occasional, 699 moderate and 656 heavy alcohol intake participants.

Difference across groups, *** P<0.0007

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Supplemental Figure 1: Plots of methionine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid concentrations as a function of tryptophan or pyridoxal 5-phosphate concentrations in combined male and female healthy young adults of the Trinity Student Study cohort.

To construct X axis coordinates, pyridoxal 5-phosphate and tryptophan concentration data were first categorized into groups in ascending order of deciles. Mean values within each decile were then calculated as X coordinates for the plot. To obtain Y coordinates, the mean (SEM) values of Y variables within each X decile were calculated. Each Y point on the graph therefore represents 22 to 24 participants (approximately one tenth of the total cohort of 2,436 participants). The fitted line was constructed using cubic spline or linear regression models to fit the X and Y coordinate data (PRISM Version 5, GraphPad Inc; www.graphpad.com/scientific-software/prism/)