

# Comparison of Age and Gender Differences of Tryptophan Metabolites in Patients of Major Monopolar and Bipolar Depression

Hiroi Tomioka<sup>1,2</sup>, Junichi Masuda<sup>3</sup>, Akikazu Takada<sup>4\*</sup>, Akira Iwanami<sup>1</sup>

<sup>1</sup>Department of Psychiatry, Showa University School of Medicine, Showa, Japan

<sup>2</sup>Department of Psychiatry, Showa University Northern Yokohama Hospital, Showa, Japan

<sup>3</sup>Global Application Development Center, Shimadzu Corporation, Kanagawa-ken, Japan

<sup>4</sup>International Projects on Food and Health (NPO), Tokyo, Japan

Email: \*takadaa@mwd.biglobe.ne.jp

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## Abstract

**Background:** No research has been done for the determination of plasma levels of tryptophan metabolites in patients of monopolar and bipolar depression. **Methods:** Ultra high-speed liquid chromatography/mass spectrometry has been used for the simultaneous determination of plasma levels of tryptophan metabolites in depressive patients. **Results:** No significant age and gender differences were shown in monopolar depressive patients and some differences were shown in bipolar patients. The administration of drugs such as antidepressants, antipsychotics, mood stabilizers do not seem to have affected the results. **Conclusion:** In patients of major monopolar depression age and gender differences of plasma levels of tryptophan metabolites disappear although significant differences are observed in healthy volunteers. Some differences of age and gender differences were shown between monopolar and bipolar depressive patients.

## Keywords

Depression, Monopolar Depression, Bipolar Depression, Tryptophan, Serotonin, 5-Hydroxyindole Acetic Acid, Kynurenine, 3-Hydroxykynurenine, Kynurenic Acid, Anthranilic Acid, Xanthurenic Acid, Indole-3-Acetic Acid, SSRI (Selective Serotonin Reuptake Inhibitor), SNRI (Serotonin Norepinephrine Reuptake Inhibitor), Anxiolytic, Antipsychotic

## 1. Introduction

22.8% of the global burden of diseases is due to psychiatric disorder [1]. Depres-

sion is considered to be the leading cause of this disability. The number of people suffering from depression substantially increased since 1990, probably due to increase in aging people [2].

Antidepressants are widely used for the treatment of major depression. However, there has been a long lasting debate and concern about their efficacy and effectiveness. Generally, clinicians have a wide choice of individual drugs and they need evidence to make the best choice for patients.

Using a meta-analysis of data of the effectiveness of antidepressants, it has recently been shown that all antidepressants are more effective than placebo in adults with major depressive disorders [3].

Serotonin, one of important metabolites of TRP has been implicated to play roles of patho-etiology of depression, there debates about roles of serotonin.

Some people doubt roles of 5-HT in etiology of depression because 5-HT concentration was not decreased in the brains of depressive patients [4].

We have recently succeeded in the simultaneous measurements of all the TRP metabolites in plasma [5] [6] [7] [8]. Using this technology we measured all TRP metabolites in plasma of depressive patients.

We analyzed plasma levels of patients of major monopolar depression and preliminarily reported that plasma levels of 5-HT, but not 5-HIAA, were lower in depressive patients [6].

However, since there are significant gender and age differences in plasma levels of TRP metabolites in healthy volunteers, we decided to analyze differences of TRP metabolites in patients of major monopolar depression and bipolar depression.

## 2. Methods

### 1) Patients

Outpatients of depression were recruited in this study. Patients were diagnosed using the criteria of Diagnostic and Statistical Manual of Mental Disorders, the 5th edition (DSM-5, American Psychiatric Association 2013) [9]. Fasting blood samples were taken early in the morning. Their severity of depression was checked by CGI-S [10], SDS [11], and HDR-S [12]. The history of prescriptions of drugs such as antidepressants, anxiolytics, mood stabilizers and other drugs were asked.

Sample numbers are 55 (male; 15, and female 40, and average age;  $45.4 \pm 11.9$ ). The number of MDD is 36 and BD is 19. Further characteristics of patients are described below. Outpatients who showed symptoms of depression were recruited in the studies at the Department of Psychiatry of Showa University Northern Yokohama Hospital. Patients were required to have had no lifetime history of psychosis, personality disorder, or drug or alcohol misuse. We also excluded patients who had organic problems of the brains. This study was carried out from 2016 to 2019. All patients gave their consent in a written form after receiving comprehensive information on the study protocol.

## 2) Measurements of TRP metabolites.

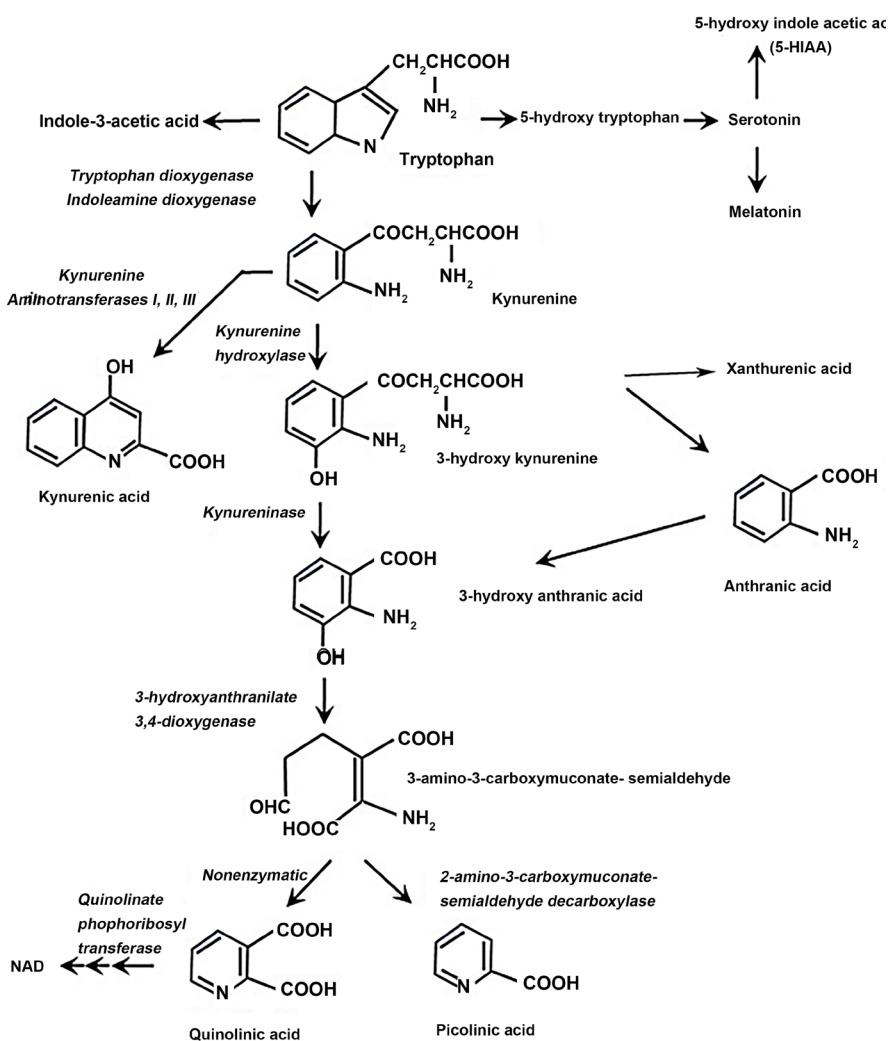
Plasma factors were measured after plasma was separated from blood (3000 rpm/min. at 4°C). Ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant.

The simultaneous measurements of TRP metabolites in plasma were performed by using an ultrahigh-speed liquid chromatography/spectrometry. Detailed methodology was described elsewhere [5] [6] [7] [8] [13]. TRP metabolic pathways are shown in **Figure 1**.

**Figure 1** shows how each metabolite of TRP is located in the map.

### Statistics

Statistical significance of the differences between groups was calculated according to one-way ANOVA. When ANOVA indicated a significant difference ( $P < 0.05$ ), the mean values of the treatment were compared using Tukey's least



**Figure 1.** Metabolic pathway of TRP. There are three TRP metabolic pathways. Indole-3-acetic acid, serotonin and kynurenine pathways [7].

significant difference test at  $P < 0.05$ . The statistical comparison between groups is analyzed by the two-way ANOVA. Mann-Whitney U test was also used for evaluating statistical significance of the use of drugs.

### 3. Results

1) Tryptophan metabolites of patients with MDD.

**Table 1** shows that by using one way ANOVA tests in patients of MDD only KYN levels of young men were higher than young women and old men. There are no significant difference in other metabolites among 4 groups.

**Table 2** shows that KYN level of old men was higher that of other 3 groups and 5-HIAA level was higher that other 3 groups. The level of KYNA of old men was higher that that of young women.

**Table 3** shows following results.

The results of two-way ANOVA were as follows:

\*1 Age,

Diagnosis:  $F(1, 47) = 0.298, p = 0.588$ ; Sample name:  $F(3, 47) = 15.598, p < 0.01$ ; and Diagnosis  $\times$  Sample name interaction:  $F(3, 47) = 0.754, p = 0.526$ .

\*2 Height,

Diagnosis  $\times$  Sample name interaction:  $F(3, 47) = 2.991, p < 0.05$ .

\*3 Weight,

Diagnosis:  $F(1, 47) = 3.358, p = 0.074$ ; Sample name:  $F(3, 47) = 7.195, p < 0.01$ ; and Diagnosis  $\times$  Sample name interaction:  $F(3, 47) = 0.961, p = 0.419$ .

\*4 CGI-S,

Diagnosis  $\times$  Sample name interaction:  $F(3, 47) = 3.585, p < 0.05$ .

\*5 SDS,

Diagnosis:  $F(1, 41) = 0.003, p = 0.955$ ; Sample name:  $F(3, 41) = 3.599, p < 0.05$ ; and Diagnosis  $\times$  Sample name interaction:  $F(3, 41) = 0.661, p = 0.581$ .

\*6 HDR-S,

Diagnosis  $\times$  Sample name interaction:  $F(3, 47) = 7.155, p < 0.01$ .

\*7 Tryptophan (TRP),

Diagnosis:  $F(1, 47) = 0.070, p = 0.793$ ; Sample name:  $F(3, 47) = 4.131, p < 0.05$ ; and Diagnosis  $\times$  Sample name interaction:  $F(3, 47) = 1.962, p = 0.133$ .

\*8 Kynurenine (KYN),

Diagnosis  $\times$  Sample name interaction:  $F(3, 47) = 6.538, p < 0.01$ .

\*9 5-Hydroxyindoleacetic acid (5-HIAA),

Diagnosis  $\times$  Sample name interaction:  $F(3, 47) = 3.129, p < 0.05$ .

\*10 Kynurenic acid,

Diagnosis  $\times$  Sample name interaction:  $F(3, 47) = 7.405, p < 0.01$ .

\*11 Xanthurenic acid,

Diagnosis  $\times$  Sample name interaction:  $F(3, 47) = 3.222, p < 0.005$ .

The ratios of products per substrate (TRP) indicate the rates of the production of the products. **Table 4** shows that there was no significant difference in the rates of the production of the products from TRP in patients of MDD and BD.

**Table 1.** Tryptophan metabolites of patients with MDD.

Diagnosis	MDD n = 38				significant differences
	young men n = 7 a	young women n = 18 b	old men n = 3 c	old women n = 10 d	
Age (years)	36.00 ± 9.256	40.00 ± 7.004	60.00 ± 14.73	59.40 ± 7.306	a vs c: <i>p</i> < 0.01 a vs d: <i>p</i> < 0.01 b vs c: <i>p</i> < 0.01 b vs d: <i>p</i> < 0.01 b vs d: <i>p</i> < 0.01
Height (m)	1.72 ± 0.07	1.59 ± 0.06	1.64 ± 0.10	1.59 ± 0.05	a vs b: <i>p</i> < 0.01 a vs d: <i>p</i> < 0.01
Weight (kg)	74.41 ± 12.86	55.92 ± 7.670	58.95 ± 16.05	54.52 ± 8.389	a vs b: <i>p</i> < 0.01 a vs d: <i>p</i> < 0.01
BMI	24.97 ± 3.998	22.31 ± 4.051	22.51 ± 2.772	21.58 ± 2.982	-
CGI-S	4.429 ± 0.535	3.667 ± 0.907	4.667 ± 0.577	3.700 ± 0.483	-
SDS	62.43 ± 6.754	45.00 ± 13.31	58.00 ± 1.414	52.22 ± 6.399	a vs b: <i>p</i> < 0.01
HDR-S	21.29 ± 3.817	16.94 ± 5.150	29.67 ± 5.033	18.30 ± 4.398	b vs c: <i>p</i> < 0.01 c vs d: <i>p</i> < 0.01
ID2: Tryptophan (µM)	64.09 ± 13.77	58.48 ± 10.40	59.11 ± 2.026	51.24 ± 11.01	-
ID3: L-5-Hydroxytryptophan (nM)	11.26 ± 3.447	9.191 ± 2.942	1.1800000	8.106 ± 6.660	-
ID4: Serotonin (nM)	325.6 ± 351.4	267.6 ± 204.4	120.3 ± 96.01	160.2 ± 145.2	-
ID5: Kynurenine (nM)	2506.5 ± 1042.9	1653.0 ± 510.16	1014.2 ± 418.25	1666.4 ± 534.60	a vs b: <i>p</i> < 0.05 a vs c: <i>p</i> < 0.05
ID6: 5-Hydroxytryptophol (nM)	-	10.50	25.11	2.20	-
ID7: Tryptophol (nM)	2.2950000	12.00 ± 18.10	-	11.99 ± 11.18	-
ID8: 5-Hydroxyindoleacetic acid (nM)	28.04 ± 22.07	35.00 ± 28.99	22.82 ± 28.11	48.68 ± 58.22	-
ID9 :Indole-3-acetic acid (nM)	2631.3 ± 1666.0	2068.4 ± 1525.8	583.0 ± 266.4	2412.5 ± 1026.1	-
ID10: Anthranilic acid (nM)	5.61	14.29 ± 11.76	-	13.89 ± 21.11	-
ID11: Kynurenic acid (nM)	67.07 ± 39.75	32.09 ± 15.37	26.47 ± 12.76	36.57 ± 14.38	-
ID12: Quinaldic acid (nM)	10.14 ± 7.55	7.46 ± 3.27	-	7.81 ± 3.96	-
ID13: 3-Indolebutyric acid (nM)	18.93 ± 12.54	18.45 ± 11.93	2.92 ± 4.04	25.62 ± 20.27	-
ID14: 3-Hydroxykynurenine (nM)	19.03 ± 14.17	20.55 ± 11.06	29.96 ± 8.09	13.98 ± 5.98	-
ID15: 3-hydroxyanthranilic acid (nM)	27.37 ± 11.08	28.12 ± 21.21	-	26.85 ± 14.18	-
ID16: Xanthurenic acid (nM)	19.50 ± 11.06	10.12 ± 6.17	11.88 ± 1.91	13.05 ± 5.79	-
ID17: Melatonin (nM)	0.17	6.61 ± 8.26	-	25.85 ± 36.24	-
ID19: Quinolinic acid (positive) (nM)	776.1 ± 1091.6	404.6 ± 277.7	168.5 ± 5.9	334.1 ± 303.9	-

One-way ANOVA was used for evaluating statistical significance. A, b, c, d indicate values of young and old men and women.

**Table 4** shows that 5-HIAA/TRP ratio was higher in BD patients than MDD patients.

**Table 5** shows that the presence of old men resulted in increase in 5-HIAA/TRP ratio.

2-2; Influences of drug administrations on substrate product ratios.

**Table 2.** Tryptophan metabolites of patients with BD.

Diagnosis	BD n = 17				significant differences
	young men n = 2 a	young women n = 10 b	old men n = 3 c	old women n = 2 d	
Age (years)	40.50 ± 0.707	39.40 ± 8.140	58.33 ± 4.619	51.00 ± 0.000	b vs c: <i>p</i> < 0.01 b vs d: <i>p</i> < 0.01
Height (m)	1.63 ± 0.00	1.58 ± 0.04	1.73 ± 0.04	1.56 ± 0.01	b vs c: <i>p</i> < 0.01 c vs d: <i>p</i> < 0.01
Weight (kg)	73.00 ± 9.899	59.34 ± 2.215	74.17 ± 7.522	60.30 ± 3.677	a vs b: <i>p</i> < 0.05 b vs c: <i>p</i> < 0.01 c vs d: <i>p</i> < 0.05
BMI	27.48 ± 3.726	23.69 ± 1.371	25.02 ± 3.572	24.77 ± 1.062	-
CGI-S	4.000 ± 0.000	4.100 ± 0.738	3.000 ± 1.000	4.000 ± 0.000	-
SDS	63.00 ± 4.243	51.56 ± 7.986	44.33 ± 8.387	55.00 ± 7.071	-
HDR-S	18.00 ± 0.000	17.70 ± 5.012	11.00 ± 2.000	15.00 ± 2.828	-
ID2: Tryptophan (µM)	57.71 ± 2.006	61.68 ± 16.96	78.50 ± 10.30	39.66 ± 11.91	-
ID3: L-5-Hydroxytryptophan (nM)	-	7.598 ± 0.886	1.2260000	-	-
ID4: Serotonin (nM)	302.0 ± 45.28	459.7 ± 402.5	250.3 ± 184.6	200.5 ± 128.0	-
ID5: Kynurenine (nM)	1119.0 ± 161.08	1389.8 ± 342.36	2426.2 ± 628.86	914.89 ± 73.22	a vs c: <i>p</i> < 0.05 b vs c: <i>p</i> < 0.01 c vs d: <i>p</i> < 0.01
ID6: 5-Hydroxytryptophol (nM)	-	-	-	-	-
ID7: Tryptophol (nM)	-	-	-	-	-
ID8: 5-Hydroxyindoleacetic acid (nM)	10.62 ± 6.362	17.01 ± 13.09	29.83 ± 7.324	116.83 ± 27.63	a vs d: <i>p</i> < 0.01 b vs d: <i>p</i> < 0.01 c vs d: <i>p</i> < 0.01
ID9 :Indole-3-acetic acid (nM)	852.8 ± 418.1	1500.7 ± 959.3	835.1 ± 217.5	1184.4 ± 1195.2	-
ID10: Anthranilic acid (nM)	-	11.59	-	-	-
ID11: Kynurenic acid (nM)	24.87 ± 14.39	41.91 ± 25.76	113.43 ± 60.29	24.55 ± 18.43	b vs c: <i>p</i> < 0.05
ID12: Quinaldic acid (nM)	-	9.97 ± 4.84	1.99 ± 0.86	-	-
ID13: 3-Indolebutyric acid (nM)	4.39 ± 0.49	19.94 ± 18.23	2.95 ± 0.00	5.72 ± 1.80	-
ID14: 3-Hydroxykynurenine (nM)	28.66 ± 0.35	29.00 ± 16.64	25.91 ± 9.04	10.21 ± 13.06	-
ID15: 3-hydroxyanthranilic acid (nM)	0.52 ± 0.00	19.91 ± 21.48	18.15 ± 14.70	-	-
ID16: Xanthurenic acid (nM)	7.65 ± 7.65	16.48 ± 13.72	30.84 ± 13.55	9.75	-
ID17: Melatonin (nM)	-	-	-	-	-
ID19: Quinolinic acid (positive) (nM)	168.2 ± 2.8	259.2 ± 200.1	227.7 ± 96.9	134.3 ± 13.3	-

One-way ANOVA was used for evaluating statistical significance. A,b,c,d indicate values of young and old men and women.

**Table 6** shows that using U test, KYN/TRP ratio was higher in patients who took SSRI/SNRI.

#### 4. Discussion

TRP is an essential amino acid needed not only for the protein synthesis but its metabolites have important functions in many physiological and pathological

**Table 3.** Comparison of tryptophan metabolites between MDD and BD.

Diagnosis	MDD n = 38				BD n = 17				significant differences
	young men n = 7 a	young women n = 18 b	old men n = 3 c	old women n = 10 d	young men n = 2 a	young women n = 10 b	old men n = 3 c	old women n = 2 d	
Age (years)	36.00 ± 9.256	40.00 ± 7.004	60.00 ± 14.73	59.40 ± 7.306	40.50 ± 0.707	39.40 ± 8.140	58.33 ± 4.619	51.00 ± 0.000	*1
Height (m)	1.72 ± 0.07	1.59 ± 0.06	1.64 ± 0.10	1.59 ± 0.05	1.63 ± 0.00	1.58 ± 0.04	1.73 ± 0.04	1.56 ± 0.01	*2
Weight (kg)	74.41 ± 12.86	55.92 ± 7.670	58.95 ± 16.05	54.52 ± 8.389	73.00 ± 9.899	59.34 ± 2.215	74.17 ± 7.522	60.30 ± 3.677	*3
BMI	24.97 ± 3.998	22.31 ± 4.051	22.51 ± 2.772	21.58 ± 2.982	27.48 ± 3.726	23.69 ± 1.371	25.02 ± 3.572	24.77 ± 1.062	-
CGI-S	4.429 ± 0.535	3.667 ± 0.907	4.667 ± 0.577	3.700 ± 0.483	4.000 ± 0.000	4.100 ± 0.738	3.000 ± 1.000	4.000 ± 0.000	*4
SDS	62.43 ± 6.754	45.00 ± 13.31	58.00 ± 1.414	52.22 ± 6.399	63.00 ± 4.243	51.56 ± 7.986	44.33 ± 8.387	55.00 ± 7.071	*5
HDR-S	21.29 ± 3.817	16.94 ± 5.150	29.67 ± 5.033	18.30 ± 4.398	18.00 ± 0.000	17.70 ± 5.012	11.00 ± 2.000	15.00 ± 2.828	*6
ID2: Tryptophan (µM)	64.09 ± 13.77	58.48 ± 10.40	59.11 ± 2.026	51.24 ± 11.01	57.71 ± 2.006	61.68 ± 16.96	78.50 ± 10.30	39.66 ± 11.91	*7
ID3: L-5-Hydroxytryptophan (nM)	11.26 ± 3.447	9.191 ± 2.942	1.1800000	8.106 ± 6.660	-	7.598 ± 0.886	1.2260000	-	-
ID4: Serotonin (nM)	325.6 ± 351.4	267.6 ± 204.4	120.3 ± 96.01	160.2 ± 145.2	302.0 ± 45.28	459.7 ± 402.5	250.3 ± 184.6	200.5 ± 128.0	-
ID5: Kynurenine (nM)	2506.5 ± 1042.9	1653.0 ± 510.16	1014.2 ± 418.25	1666.4 ± 534.60	1119.0 ± 161.08	1389.8 ± 342.36	2426.2 ± 628.86	914.89 ± 73.22	*8
ID6: 5-Hydroxytryptophol (nM)	-	10.50	25.11	2.20	-	-	-	-	-
ID7: Tryptophol (nM)	2.2950000	12.00 ± 18.10	-	11.99 ± 11.18	-	-	-	-	-
ID8: 5-Hydroxyindoleacetic acid (nM)	28.04 ± 22.07	35.00 ± 28.99	22.82 ± 28.11	48.68 ± 58.22	10.62 ± 6.362	17.01 ± 13.09	29.83 ± 7.324	116.83 ± 27.63	*9
ID9: Indole-3-acetic acid (nM)	2631.3 ± 1666.0	2068.4 ± 1525.8	583.0 ± 266.4	2412.5 ± 1026.1	852.8 ± 418.1	1500.7 ± 959.3	835.1 ± 217.5	1184.4 ± 1195.2	-
ID10: Anthranilic acid (nM)	5.61	14.29 ± 11.76	-	13.89 ± 21.11	-	11.59	-	-	-
ID11: Kynurenic acid (nM)	67.07 ± 39.75	32.09 ± 15.37	26.47 ± 12.76	36.57 ± 14.38	24.87 ± 14.39	41.91 ± 25.76	113.43 ± 60.29	24.55 ± 18.43	*10
ID12: Quinaldic acid (nM)	10.14 ± 7.55	7.46 ± 3.27	-	7.81 ± 3.96	-	9.97 ± 4.84	1.99 ± 0.86	-	-
ID13: 3-Indolebutyric acid (nM)	18.93 ± 12.54	18.45 ± 11.93	2.92 ± 4.04	25.62 ± 20.27	4.39 ± 0.49	19.94 ± 18.23	2.95 ± 0.00	5.72 ± 1.80	-
ID14: 3-Hydroxykynurenine (nM)	19.03 ± 14.17	20.55 ± 11.06	29.96 ± 8.09	13.98 ± 5.98	28.66 ± 0.35	29.00 ± 16.64	25.91 ± 9.04	10.21 ± 13.06	-
ID15: 3-hydroxyanthranilic acid (nM)	27.37 ± 11.08	28.12 ± 21.21	-	26.85 ± 14.18	0.52 ± 0.00	19.91 ± 21.48	18.15 ± 14.70	-	-
ID16: Xanthurenic acid (nM)	19.50 ± 11.06	10.12 ± 6.17	11.88 ± 1.91	13.05 ± 5.79	7.65 ± 7.65	16.48 ± 13.72	30.84 ± 13.55	9.75	*11
ID17: Melatonin (nM)	0.17	6.61 ± 8.26	-	25.85 ± 36.24	-	-	-	-	-
ID19: Quinolinic acid (positive) (nM)	776.1 ± 1091.6	404.6 ± 277.7	168.5 ± 5.9	334.1 ± 303.9	168.2 ± 2.8	259.2 ± 200.1	227.7 ± 96.9	134.3 ± 13.3	-

Two-way ANOVA with diagnosis (MDD and BD) and sample name (young and old men and women) was used for evaluating statistical significance.

processes [14]. Humans do not synthesize TRP, which must be taken from diet. The required daily dose is considered to be 3.5 mg per kg of weight [15]. Less than 1% of ingested TRP is used for protein synthesis and the majority of TRP is metabolized along three known pathways, Indole-3-acetic acid (IAA), 5-HT and KYN pathways. The majority of TRP enters from the gut to portal circulation and metabolized in the liver. The remaining TRP, together with liver degradation products, transported to the brain, heart and skeletal muscle.

Gut microbiota play important roles in absorbing TRP and produce indoles, which act as hydroxyl radical scavengers, neuroprotectants, and human AhR (aryl hydrocarbon receptor) selective agonists attenuating inflammations [16].

**Table 4.** Comparison of ratios of tryptophan metabolites per tryptophan. (a) Results of two way ANOVA tests for monopolar major depressive patients; (b) Results of two way ANOVA tests for bipolar depressive patients.

(a)					
Diagnosis	MDD n = 38				significant differences
Sample name	young men n = 7 a	young women n = 18 b	old men n = 3 c	old women n = 10 d	
5-HTRP/TRP	0.18 ± 0.10	0.16 ± 0.05	0.02	0.15 ± 0.13	
serotonin/TRP	5.24 ± 5.24	4.47 ± 3.24	2.02 ± 1.58	3.19 ± 3.06	-
5-HIAA/TRP	0.48 ± 0.46	0.65 ± 0.64	0.38 ± 0.46	0.92 ± 0.91	-
KYN/TRP	42.19 ± 23.68	28.66 ± 8.23	17.06 ± 6.65	33.65 ± 12.12	-

(b)					
Diagnosis	BD n = 17				significant differences
Sample name	young men n = 2 a	young women n = 10 b	old men n = 3 c	old women n = 2 d	
5-HTRP/TRP	-	0.16 ± 0.04	0.02	-	
serotonin/TRP	5.25 ± 0.97	7.52 ± 6.48	3.15 ± 2.07	5.80 ± 4.97	-
5-HIAA/TRP	0.18 ± 0.10	0.32 ± 0.30	0.38 ± 0.07	3.19 ± 1.66	-
KYN/TRP	19.45 ± 3.47	23.94 ± 8.74	31.27 ± 8.72	23.87 ± 5.32	-

**Table 5.** Comparison of tryptophan metabolites between MMD and BD.

Diagnosis	MDD n = 38				BD n = 17				significant differences
	young men n = 7 a	young women n = 18 b	old men n = 3 c	old women n = 10 d	young men n = 2 a	young women n = 10 b	old men n = 3 c	old women n = 2 d	
5-HTRP/TRP	0.18 ± 0.10	0.16 ± 0.05	0.02	0.15 ± 0.13	-	0.16 ± 0.04	0.02	-	-
serotonin/TRP	5.24 ± 5.24	4.47 ± 3.24	2.02 ± 1.58	3.19 ± 3.06	5.25 ± 0.97	7.52 ± 6.48	3.15 ± 2.07	5.80 ± 4.97	-
5-HIAA/TRP	0.48 ± 0.46	0.65 ± 0.64	0.38 ± 0.46	0.92 ± 0.91	0.18 ± 0.10	0.32 ± 0.30	0.38 ± 0.07	3.19 ± 1.66	*
KYN/TRP	42.19 ± 23.68	28.66 ± 8.23	17.06 ± 6.65	33.65 ± 12.12	19.45 ± 3.47	23.94 ± 8.74	31.27 ± 8.72	23.87 ± 5.32	-

The results of two-way ANOVA were as follows: \*5-HIAA/TRP, Diagnosis × Sample name interaction:  $F(3, 47) = 7.409, p < 0.01$ .

Since we have not done any research about roles of gut microbiota in TRP metabolism, I do not further comment on this issue.

For many people TRP is best known for a substance to generate 5-HT and melatonin [17]. Our results will be shown and discussed later. Since 5-HT is very important in the regulation of mood, anxiety and cognition, people tend to underestimate roles of KYN as a metabolite of TRP. In mammals, the majority of free TRP is degraded through KYN pathway and many metabolites are generated in inflammation, immune responses and neurotransmission [18]. Metabolites



**Table 6.** Comparison of tryptophan metabolites between MDD and BD.

	Patients took SSRI or SNRI n = 16	Patients don't took SSRI or SNRI n = 39	Mann-Whitney U	z	p
Age (years)	45.75 ± 13.27	45.28 ± 11.44	290.500	-0.399	0.690
Hight (m)	162.08 ± 7.84	161.42 ± 7.35	263.000	-0.909	0.363
Weight (kg)	60.76 ± 8.08	60.75 ± 11.91	250.000	-0.556	0.579
BMI	23.28 ± 3.76	23.22 ± 3.44	268.000	-0.192	0.848
CGI-S	3.88 ± 0.81	3.90 ± 0.79	304.500	-0.158	0.875
SDS	51.93 ± 12.62	51.53 ± 10.40	243.000	-0.261	0.794
HDR-S	18.94 ± 5.32	17.92 ± 5.70	268.500	-0.808	0.419
Tryptophan (µM)	56.98 ± 13.00	59.65 ± 13.72	298.000	-0.259	0.795
L-5-Hydroxytryptophan (nM)	8.13 ± 5.08	8.60 ± 4.27	68.500	-0.198	0.843
Serotonin (nM)	215.13 ± 249.99	311.18 ± 275.84	112.000	-1.246	0.213
Kynurenine (nM)	1856.63 ± 596.73	1603.71 ± 727.67	226.500	-1.584	0.113
5-Hydroxytryptophol (nM)	0.00 ± 0.00	12.60 ± 11.60	-	-	-
Tryptophol (nM)	24.73 ± 12.85	2.42 ± 3.57	0.000	-2.236	<0.05
5-Hydroxyindoleacetic acid (nM)	39.05 ± 47.94	32.60 ± 31.35	272.000	-0.741	0.459
Indole-3-acetic acid (nM)	2212.73 ± 1454.54	1736.09 ± 1275.02	234.000	-1.445	0.148
Anthranilic acid (nM)	15.84 ± 22.44	9.60 ± 7.95	14.000	-0.183	0.855
Kynurenic acid (nM)	39.45 ± 18.07	44.08 ± 35.54	284.000	-0.519	0.604
Quinaldic acid (nM)	9.42 ± 5.76	6.85 ± 3.70	64.500	-0.935	0.350
3-Indolebutyric acid (nM)	19.27 ± 10.72	17.81 ± 17.16	216.000	-1.428	0.153
3-Hydroxykynurenine (nM)	19.90 ± 11.97	22.05 ± 12.75	272.000	-0.741	0.459
3-hydroxyanthranilic acid (nM)	24.45 ± 16.26	24.96 ± 18.99	183.000	-0.512	0.609
Xanthurenic acid (nM)	13.05 ± 5.77	14.52 ± 11.25	271.000	-0.276	0.782
Melatonin (nM)	20.65 ± 31.35	5.38 ± 8.91	5.000	-0.866	0.386
Quinolinic acid (positive) (nM)	379.50 ± 270.30	376.81 ± 531.39	246.000	-0.969	0.333
5-HTRP/TRP	0.13 ± 0.08	0.16 ± 0.09	55.000	-0.962	0.336
serotonin/TRP	3.70 ± 4.19	5.22 ± 4.39	110.000	-1.302	0.193
5-HIAA/TRP	0.70 ± 0.78	0.65 ± 0.84	255.000	-1.056	0.291
KYN/TRP	33.57 ± 11.58	27.73 ± 13.35	205.000	-1.983	<0.05

Mann-Whitney U test was used for evaluating statistical significance. KYN/TRP ratio was higher in patients who took SSRI/SNRI ( $p < 0.05$ ).

of KYN pathway have been linked to several psychiatric and mental health disorders such as depression and schizophrenia [19]. Sixty percent of KYN are transported from the periphery through blood brain barrier (BBB) and the rest is produced locally. Stress increases many TRP metabolites [20] but these metabolites have antagonizing actions. QA is an N-methyl-p-aspartate receptor agonist and KNA is an NMDAR and  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$ nAChR) an-

tagonist. The balance of the levels of these agonist and antagonist is shown to be dysregulated in depression and schizophrenia [21]

Although the concentration of 5-HT has been considered to be low in depressive patients [22], 5-HT concentration in the brains of suicide victims is not low [4]. Therefore, it is not known if 5-HT concentration is decreased in the brains of depressive patients.

By using an ultra high-speed LC/MS, we wanted to know changes in plasma levels of TRP metabolites, especially 5-HT and 5-HIAA (6). Although plasma serotonin levels and 5-HT/TRP ratio were low in depressive patients, the levels of 5-HIAA/TRP were not lower in depressive patients. This result indicates that 5-HT is degraded to 5-HIAA in depressive patients almost to the same extent to healthy old and young women.

We measured the levels of KYN in healthy old men and young women, and depressive patients. TRP seems to be degraded to KYN pathways to the same extent in these three groups.

These results suggest that in depressive patients 5-HT was quickly degraded to 5-HIAA, and this seems to be a reason of low 5-HT levels in depressive patients.

Recently we found that there are significant gender and age differences in plasma levels of TRP metabolites [23]. Plasma levels of 5-HT, 5-HIAA, KYN, AA are higher in young women and old men than young men and old women, but plasma levels of 3-HKYN and XA are higher in young men and old women than young women and old men.

These results mean that there are significant age and gender difference in plasma levels of TRP metabolites.

We then measured plasma levels of TRP metabolites in MMD and BD and found that such significant gender and age difference disappear in MMD patients. Although some changes exist in BD patients, they are not so significant as shown in healthy people.

It is not probable that such changes are due to use of drugs for depression.

Probably in patients of depression severe hormonal change may influence TRP metabolism.

As to a relationship between serotonin pathway and KYN pathway, Lapin IP suggested that in depression tryptophan 2,3-dioxygenase in the liver shunted metabolism of serotonin away from 5-HT production to KYN production, resulting in serotonin deficiency [24]. KYN, QN and 3-HKN were shown to be anxiogenic and KNYA were anxiolytic [25]. From these results, he tried to explain the effects of anti-depressive drugs.

Our results do not support this hypothesis. Metabolites of KYN pathways were not high in depressive patients.

There have not been enough studies as to 5-HT levels in the brain of patients of bipolar depression. Serotonin levels in cerebrospinal fluids of patients of bipolar depression were shown to be high [26] or normal [27]. So, it seems to be very important to discriminate monopolar and bipolar depression to study roles of serotonin in the pathogenesis of disease.

Our results show that plasma 5-HT levels were low and metabolites of KYN pathway were not changed in patients of monopolar depression.

Comparison of TRP metabolites in healthy people and MMD or BD patients will be reported soon.

Finally, relationship of TRP metabolites levels between brain and plasma should be discussed.

It is well known that 5-HT does not pass the blood brain barrier [28]. It has been suggested that the integrity of the endothelial cell lining of the cerebrovascular bed constitutes a morphological blood-brain barrier mechanism to neurotransmitter monoamines. Circulating monoamines are prevented from entering the brain primarily at the luminal membrane of the endothelial lining.

So, we wonder whether the measurements of TRP levels in the blood may represent their changes in the brain.

We have reported changes in TRP metabolites levels in the brain and plasma after the application of electric shocks in rats [29] [30] [31] [32]. Most of TRP metabolites including 5-HT and 5-HIAA in the brain and plasma increased immediately after electric shock and returned to normal 30 min. later. These results suggest that plasma levels of TRP metabolites may represent those in the brain, of which the reasons we could not speculate. However, we can get some metabolic informations in the brain by analyzing plasma levels of TRP metabolites such as 5-HT, 5-HIAA, or KYN.

## **Ethics**

This work has been approved by Institutional Review Board of Showa University Northern Yokohama Hospital (No. 1508-11) and has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments.

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Experiments were designed and performed by all of the authors. AT wrote a manuscript. Statistical analyses were done by MH. All authors read the manuscript and approve the final manuscript. All the authors had responsibilities for a final content. There is no conflict of interest for any author.

## **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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### Abbreviation

tryptophan (TRP), serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA), indole-3-acetic acid (IAA), kynurenine (KYN), xanthurenic acid (XA), anthranilic acid (AA), kynurenic acid (KNA), 3-hydroxykynurenine (3-HKN), indoleamine dioxygenase (IDO), tryptophan dioxygenase (TDO), Selective serotonin uptake inhibitor (SSRI), serotonin epinephrine reuptake inhibitor (SNRI), clinical global impression-severity scale (CGI-s), self-rating depression scale (SDS), Hamilton depression rating scale(HDRS).