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Obesity: Open Access

ISSN 2380-5528 | Open Access

RESEARCH ARTICLE

Volume 4 - Issue 2 | DOI: http://dx.doi.org/10.16966/2380-5528.138

Plasma Levels of Tryptophan Metabolites in Healthy Young and Old Men and Women, and Patients of Type 2 Diabetes Mellitus (T2DM)

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Received: 05 Jun, 2018 | **Accepted:** 04 Jul, 2018 | **Published:** 11 Jul, 2018

Citation: Shimizu F, Ishii Y, Ogawa M, Takao T, Matsuoka K, et al. (2018) Plasma Levels of Tryptophan Metabolites in Healthy Young and Old Men and Women, and Patients of Type 2 Diabetes Mellitus (T2DM). Obes Open Access 4(2) : dx.doi.org/10.16966/2380-5528.138

Abstract

Background: Plasma levels of tryptophan (TRP) metabolites have not been measured extensively in patients of type 2 diabetes mellitus (T2DM).

Methods: Body mass index (BMI) and plasma levels of various factors such as glucose, lipids and TRP metabolites have been measured in healthy young and old men and women, and patients of T2DM by using the ultrahigh speed liquid chromatography-mass spectroscopy (Shimadzu Corporation).

Results: There are age and gender differences in TRP metabolism. The plasma levels of 5-hydroxytryptophan (5-HTRP), 5-hydroxyindoleacetic acid (5-HIAA), kynurenic acid (KNA), 3-hydroxykynurenine (3-HKN), and 3-hydroxyanthranilic acid (3-HAA) were higher in patients of old men of T2DM than healthy old men. Since 5HTRP and 5-HIAA belong to the serotonin pathway, and KNA, 3-HKN, and 3-HAA belong to the KN pathway of TRP metabolism, these pathways were activated more in the patients of T2DM. Since plasma levels of Indole-3-acetic acid were not elevated in T2DM, that pathway was not activated more in T2DM. Although the serotonin pathway was activated more in the patients of T2DM, serotonin (5-HT;5-hydoroxytyptamine) levels were not increased compared with those in T2DM patients, which may mean that 5-HT was quickly metabolized to 5-HIAA in the patients of T2DM.

Conclusion: There are gender and age differences in TRP metabolism. Plasma levels of many tryptophan metabolites in serotonin and kynurenine pathways increased in T2DM patients compared with those in healthy old men.. Since stress increases these metabolites these results may imply that patients of T2DM are exposed to psycho somatic and oxidative stresses.

Keywords: Tryptophan; Serotonin; 5-hydroxyindole acetic acid; Kynurenine; 3-hydroxykynurenine; Kynurenic acid; Indole-3-acetic acid; 3-hydroxyanthranilic acid; Quinolinic acid; Picolinic acid; Diabetes mellitus

Introduction

Tryptophan (TRP) is an essential amino acid important for protein synthesis, but it also serves as substrate for the generation of several bioactive compounds with important physiological roles. Probably the best-known fate of TRP is its conversion to serotonin (5-HT; hydroxytryptamine), an important neurotransmitter involved in the control of many responses in the central nervous system (CNS) and linked to alterations in mood, anxiety, or cognition [1]. Serotonin can be further converted to N-acetylserotonin (NAS) and melatonin, influencing control over circadian rhythmicity, one of biological roles for TRP metabolites [2]. However, in mammals, the majority of free TRP is degraded through the kynurenine pathway (KP) and generates many metabolites involved in inflammation, immune response, and excitatory neurotransmission [3]. We have shown that foot shock applied to rats resulted in increase of not only brain 5-HT levels but KNY levels in plasma, kidney, liver and every part of the brain [4-6].

Several TRP metabolites have been shown to exhibit neuroexcitatory, convulsant, and toxic properties [7,8]. In the periphery, only 1% of dietary tryptophan was converted to serotonin and more than 95% was metabolized to kynurenines [9,10].

We thought that tryptophan metabolism may have been changed in diabetic patients because of various stresses both physiological

and psychological including self-care. So we measured plasma levels of 5-HT, 5-HIAA and kynurenine metabolites, and showed that TRP metabolites increased in plasma of patients of type 2 diabetes mellitus (T2DM) [11].

We now thought that it is important to know plasma levels of TRP metabolites of young and old men and women, and patients of T2DM because these data are critical to analyze roles of the objective of the study is to know differences in plasma levels of TRP metabolites and to compare their levels in T2DM patients young, old men and women.

Methods

We asked male and female acquaintances older than 50 years old and male and female college students to participate in the experiments. Acquaintances mean that these participants are personal friends of our group member. The sample sizes and ages of participants are as follows. Old men (n=25, age; 60.8 ± 9.9) and old women (n=39, age; 67.4 ± 7.5) and young men (n=49.age; 20.7 ± 1.5) and young women (n=47, age; 21.2 ± 0.7) Young men are students of Tokyo Institute of Technology and young women are students of Showa Women's college.

We obtained an informed consent prior to conducting the protocol which had been approved by the Ethical Committee of Showa Women's University .and Saiseikai main Hospital.

Drs. K. Matsuoka and K. Kato, who are internists checked their health carefully and examined their blood samples and recruited them if there were no health problems such as diabetes, hypertension or not serious diseases experienced in the past. They did not smoke in the past. We also excluded people who took drugs for dyslipidemia, hyperglycemia, or hypertension. Their disease histories were average 5 years. They were mainly treated by the controls of life styles such as foods intakes and exercises. Nobody used insulin for treatments. Some took metabolism promoting drugs such as metformin. Doctors went to colleges to check health of young participants. We collected blood samples early morning. Participants were asked not to eat anything after 21.00 PM the previous evening. Plasma specimens were collected for assays of blood parameters. We obtained an informed consent prior to conducting the protocol which had been approved by the Ethical Committee of Showa Women's University and Saiseikai Shibuya Satellite Clinic.

Healthy participants were given self-administered diet history questionnaires and described answers on each item by recollection of diets they took (7 days dietary recall). We used a brief-type self-administered diet history questionnaire (BDHQ) by using which the Japanese Ministry of Health, Labour and Welfare reports National Nutrition Surveys. From these questionnaires, we calculated the intakes of energy, carbohydrate, fat, and protein.

Plasma factors were measured after plasma was separated from blood. Ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant. Blood glucose levels were measured by a hexokinase UV method. Insulin was measured by the CLEIA (chemiluminescent immunoassay) method. We also measured glycemic indexes after giving glucose and sucrose to participants, so that we did not use HbA1c as a marker of glycemia.

Lipid and lipoprotein concentrations such as total cholesterol, HDL (high density lipoprotein cholesterol), LDL (low density lipoprotein) cholesterol, TG (triglyceride) were determined using a Polychem Chemistry Analyzer (Polymedco Inc.) FFA (free fatty acid) and the concentrations of ω fatty acids such as AA (arachidonic acid), DHA (docosahexanoic acid), and EPA (eicosapentanoic acid) were measured by a gas chromatography.

Remnant lipoproteins (RLPs) were isolated from the serum to an immunoaffinity mixed gel containing anti-apolipoprotein A1 and anti-apolipoprotein B100 monoclonal antibodies (Japan Immunoresearch Laboratories, Takasaki, Japan), and the cholesterol and triglyceride concentrations of the unbound fraction were measured as RLP cholesterol and RLP-triglyceride, respectively.

Fasting plasma samples of T2DM patients were obtained early morning. They were males over 50 years old. Some patients used antidiabetic drugs, but most of them were treated by keeping life styles such as food intakes in good condition. No patients used insulin for treatment.

Measurements of tryptophan metabolites (Figure 1)

Reagents

The standard materials of fifteen major tryptophan metabolites such as TRP, L-5-Hydroxytryptophan, 5-HT, L-Kynurenine (KYN), 3-Hyroxykinurenine (3-HKYN), 5-Hydroxytryptophol (5-HTOL), tryptophol (TOL), Kynurenic acid (KA), Quinaldic acid (QA), Xanthutrenic acid (XA), 5-Hydroyindole-acetic acid (HIAA), Indole-3-acetic acid (IAA), 3-Hyroxyanthranilic acid (HAA), Anthranilic acid (AA), Indole-3-lacctic acid (ILA) are special grade or biochemical grade regents purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Formic acid and acetonitrile are LC/MS grade regents and other major chemicals such as methanol are special grade purchased from Wako Chemical as well. Water was obtained as occasion demands from the installed Milli-Q water purification system (Merck-Millipore, MA, USA)

Each standard mother solution was prepared as 1000 mg/L from each regent above in water and/or 0.1 mol/L sodium hydroxide aqueous solution. Standard mixture solution for each concentration level of a calibration curve was prepared by dilution of the mother solution with water.

Instrumentation and analytical conditions

TRP metabolites were analyzed Shimadzu LCMS-8060 or 8050 system consists of Nexera UHPLC system such as solvent delivery units, an autosampler and column oven, and liquid chromatograph mass spectrometer LCMS-8060 or 8050 with its electro spray interface. (Shimadzu Corporation, Kyoto, Japan) The HPLC column used was L-column 2 ODS (2.1 mm × 150 mm, 3 μm, CERI; Chemical Evaluation and Research Institute, Tokyo, Japan) with a gradient elution of 0.1% formic acid / acetonitrile system. Before analyses, the condition of multi reaction monitoring (MRM) was optimized using each standard solution, respectively.

Gradient elution was performed by the high pressure binary gradient program, 5% of 0.1 formic acid aqueous solution fold in 3 min with acetonitrile, 5% to 95% of 0.1% formic acid aqueous solution in the next 6 min followed by 95% hold in another 3 min at flow rate 0.4 mL/min under 40°C. Ionization was executed by an electrospray (ESI) positive mode at 150°C (the desolvation line) and 400°C (the interface) with 3 L/min nebulizing and 5 L/min drying gas. Chromatographic data were obtained by MRM mode under optimized transition.

Sample pretreatment

The samples were stored at -80°C.

50 μL aliquot of the blood plasma sample was took in 1.5 ml test tube, and then 25 μL 0.1% formic acid aqueous solution, 150 μL of acetonitrile and an additional 75 μL of 0.1% formic acid aqueous solution were added, respectively. The solution was vortexed in 30

seconds followed by standing in 5 minutes under cooling temperature. After this, each sample was centrifuged at 3000 rpm under 4°C in 10 minutes. After the centrifugation, 120 µL aliquot of supernatant for each sample solution in the 1.5 mL test tube was transferred into other 1.5 mL test tube and additional 80 µL of 0.1% formic acid aqueous solution was added followed by being vortexed in 30 seconds, respectively. The final solution was 10-flod dilution from its original blood plasma sample. 1 µL of each final solution was injected into the LC/MS system by an autosampler.

Statistics

The results are presented as means ± SD. Statistical significance of the differences between groups was calculated according by one-way ANOVA. When ANOVA indicated a significant difference ($P<0.05$), the mean values were compared using Tukey's least significant difference test at $P<0.05$.

Results

Tables 1-3

Generally speaking, plasma levels of tryptophan metabolites of young women and old men are higher than those in young men and old women.

Plasma levels of tryptophan metabolites of T2DM patients are higher than those in old men except for kynurenine. and indole butyric acid.

Figures 2-5

In contrast to data of KNY, Plasma levels of KNYA were higher in T2DM patients compared to those in young women and old men and women.

Discussion

Tryptophan (TRP) has been paid attention by scientists due to its role in the production of 5-HT in the brain, which is an important transmitter regulating mood, anxiety, cognition or memory [1]. Serotonin is further converted to 5-HIAA, or N-acetyserotonin or melatonin, which controls circadian rhythmicity. However, as stated in INTRODUCTION, the majority of free TRP is degraded through the kynurenine pathway. The final product is nicotinamide adenine dinucleotide (NAD^+), which is now under investigation for a therapeutic target of several diseases [12] Kynurenine (KN) and its metabolites are also known for their actions in the central nervous system. They stimulate or suppress the functions of glia cells. Defects in KN signaling are shown in mouse model of Alzheimer disease or Huntington disease.

TRP is an essential amino acid which must be intaken from the food. Egg, fish, dairy products, meat, and legumes contain higher levels of TRP compared with other foods of vegetable origin.

There are some papers indicating that insulin regulates the metabolism of carbohydrate, lipid, protein, and amino acid [13]. Proteolysis and associated release of amino acids are inhibited by insulin and insulin stimulates amino acid uptake and protein synthesis in skeletal muscle [14,15]. High insulin levels were shown to stimulate skeletal muscle protein synthesis [16]. As to individual amino acids, we have shown that plasma levels of isoleucine, leucine, aspartic acid, glutamic acid are higher in T2DM patients and arginine, asparagine, cystine, and glutamine are lower in T2DM patients compared with those in old men [11]. It has been shown that plasma levels of phenylalanine, valine, leucine, isoleucine, and tyrosine increased and those of histidine and glutamine decreased in hyperglycemia [17]. So, the metabolism of some amino acids may be influenced in T2DM.

The majority of TRP is metabolized along the 5-HT, KNY, or indole pathways. After the absorption of amino acids from the intestine, TRP is transported by large neutral amino acid transporters.

Only a fraction of the majority of TRP imported into the gut is used. The remaining TRP is imported to the liver and then transported to tissues such as the brain, heart, and skeletal muscle.

Although TRP metabolites play important roles in the intestine, pancreas, skeletal muscle, liver or immune system, we here discuss their roles in pathogenesis of diabetes mellitus.

As stated above TRP metabolism is linked to inflammation and immune suppression. The increased production of serotonin is reported to be related to pathogenesis of diabetes [18]. Our results, however, show that plasma levels of 5-HT were not significantly increased in T2DM patients compared with those in old men (Table 2). Tryptophan hydroxylase (TRP hydroxylase) which converts TRP to 5-HTRP is known to be a rate-limiting factor [19] The increased plasma levels of 5-HRP and 5-HIAA may mean that 5-HT was rapidly formed from TRP and also rapidly metabolized to 5-HIAA in patients of T2DM.

Chronic stress and low-grade inflammation are major risk factors in prediabetes to diabetes transition. They can change the balance of TRP metabolism toward KN, 3-HK, and KNA, both by activating TDO (tryptophan 2,3 dioxygenase)/IDO (indoleamine 2,3 dioxygenase) and by reducing the availability of pyridoxal-5-phosphate, a necessary cofactor for many KP (kynurenine pathway) enzymes.

Diabetic patients show increased levels of XA (xanthurenic acid) and KNA in urine, which have been consequently suggested as biomarkers for T2DM [20-22]. Our results, however, show that although plasma levels of KNA were increased, those of XA were not elevated in patients of T2DM. Moreover, TRP metabolites inhibit both proinsulin synthesis and glucose- and leucine-induced insulin release from rat pancreatic islets, and XA in particular binds to circulating insulin and prevents its action on target cells [23]. Recently, KN-AhR (aryl hydrocarbon receptor) signaling in mice has been suggested to play a role in the etiology of obesity.

In the present research we report that there are gender and age differences in TRP metabolism (Table 3 and Figure 1) so, we must compare TRP metabolism between old men of T2DM with healthy old men. Taking age and gender differences into consideration, we showed that the degradation of TRP into the serotonin and kynurenine pathway was increased in T2DM patients, but that plasma levels of not all the metabolites increased in patients of T2DM.

These results, however, support the idea that there are increased immune and stress activities in diabetes. Quinolinic acid is an agonist of N-methyl-D- aspartate receptor and kynurenic acid is an antagonist [7,8]. Our results show that the plasma levels of KNA was increased in patients of T2DM. So it is possible that the balance of nerve agonist and antagonist is impaired in diabetes.

In fact, TRP metabolites were shown to be increased in patients of acute coronary diseases, of which the etiology acute or chronic stress is implicated [24]. In patients with suspected stable angina pectoris, elevated levels of plasma kynurenines predicted increased risk of acute myocardial infarction, and risk estimates were generally stronger in subgroups of impaired glucose homeostasis [25]. IDO activation was shown to be associated with depressive symptoms of coronary artery disease. These results may suggest that stress may activate TRP degradation and cause thrombosis in the artery.

Table 1: The background of healthy young and old men and women and T2DM patients

Subjects	Young men n=48 a	Young women n=47 b	Old men n=44 c	Old women n=39 d	T2DM (male) n=30 e	Statistical significance
Age (years)	20.7 ± 1.5	21.2 ± 0.7	62.4 ± 9.6	67.4 ± 7.5	67.0 ± 9.6	a *vs* c : p<0.01 a *vs* d : p<0.01 a *vs* e : p<0.01 b *vs* d : p<0.01 b *vs* c : p<0.01 b *vs* e : p<0.01 c *vs* d : p<0.01 c *vs* e : p<0.05
Height (m)	1.72 ± 0.06	1.58 ± 0.06	1.68 ± 0.07	1.57 ± 0.06	1.70 ± 0.06	a *vs* d : p<0.01 a *vs* b : p<0.01 a *vs* c : p<0.01 b *vs* c : p<0.01 c *vs* d : p<0.01
Weight (kg)	65.1 ± 9.2	50.9 ± 5.8	68.8 ± 10.9	50.6 ± 6.8	68.2 ± 11.5	a *vs* b : p<0.01 a *vs* d : p<0.01 b *vs* c : p<0.01 c *vs* d : p<0.01
BMI	22.1 ± 3.2	20.3 ± 1.6	24.3 ± 3.2	20.5 ± 2.5	23.3 ± 3.3	a *vs* b : p<0.05 a *vs* c : p<0.01 b *vs* c : p<0.01 b *vs* e : p<0.01 c *vs* d : p<0.01 c *vs* e : p<0.01
Energy intake (kcal/day)	1919 ± 560	1413 ± 411	2220 ± 544	1848 ± 74	-	a *vs* b : p<0.01 b *vs* c : p<0.01 b *vs* d : p<0.01 c *vs* d : p<0.05
Protein intake (g/day)	67.9 ± 23.9	51.8 ± 17.8	83.8 ± 27.4	80.0 ± 27.3	-	a *vs* b : p<0.01 b *vs* c : p<0.01 b *vs* d : p<0.01
Lipid intake (g/day)	58.8 ± 22.9	45.3 ± 13.7	63.8 ± 19.8	60.9 ± 20.9	-	a *vs* b : p<0.01 b *vs* d : p<0.01 c *vs* d : p<0.01
Carbohydrate intake (g/day)	263.9 ± 86.8	183.7 ± 62.4	268.0 ± 66.9	248.3 ± 76.9	-	a *vs* b : p<0.01 a *vs* c : p<0.01 b *vs* d : p<0.01 c *vs* d : p<0.01

One -way ANOVA was used for evaluating statistical significance. A,b,c,d indicate values of young and old men and women, and T2DM patients. P<0.05 is considered statistically significant.

As indicated before, we applied electric foot shock to rats and measured TRP metabolites in the plasma, the central nervous system and peripheral tissues [4-6]. Plasma levels of TRP increased significantly immediately after the foot shock and returned to normal values within 24 hours. TRP levels also increased in all the brain areas immediately after stress application. Foot shock elevated the levels of KN in the plasma, liver, kidney and every part of the brain. 3-HKN and KNA levels increased in the brain. These results indicate that stress activates not only serotonergic pathway but also KYN pathway in the central nervous system and periphery. Some metabolites of KYN pathway, such as 3-HKN are neurotoxic while other metabolite such as KNA is neuroprotective. Increase in 5-HT levels in the hypothalamus and midbrain stabilizes emotion and prevents mood disorders. Therefore, some brain dysfunction resulting from stress may be prevented by the metabolites of TRP. The balance of these functions may be important in the maintenance of nerve integrity and peripheral homeostasis under stress. We think that the stress may contribute to the etiology of T2DM.

Ethics

This work has been approved by the Ethical committees of Showa Women's University and NPO (non-profit organization) "International projects on food and health" and has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments.

Acknowledgments

Experiments were designed and performed by all of the authors. AT wrote a manuscript. Statistical analyses were done by TT and FS. Tryptophan metabolites were measured by Junichi Masuda, Global Application Development Center, Shimadzu Corporation. All authors read the manuscript and approved the final version. All the authors had responsibilities for the final content. No conflicts of interest for any author.

Financial support

This study was supported by grants by NPO "International Projects on Food and Health."

Table 2: Shows that the plasma levels of total cholesterol, arachidonic acid, docosahexanoic acid are higher in old menthan patients of T2DM.

Subjects	young men	young women	old men	old women	statistical significance
	n=48	n=47	n=44	n=39	
	a	b	c	d	
TG; triglyceride(mg/dL)	74.7 ± 29.9	60.4 ± 21.3	126.4 ± 81.3	88.9 ± 55.3	a *vs* c : p<0.01
					c *vs* d : p<0.01
					b *vs* c : p<0.01
HDL-Chol.; HDL-cholesterol(mg/dL)	60.3 ± 11.4	66.3 ± 8.1	60.9 ± 14.6	75.7 ± 16.5	a *vs* d : p<0.01
					b *vs* d : p<0.01
					c *vs* d : p<0.01
LDL-Chol.; LDL-cholesterol (mg/dL)	104.9 ± 23.8	87.8 ± 15.7	123.7 ± 30.2	138.6 ± 33.8	a *vs* b : p<0.01
					a *vs* c : p<0.01
					a *vs* d : p<0.01
					b *vs* c : p<0.01
					b *vs* d : p<0.01
T-Chol.; total cholesterol(mg/dL)	174.0 ± 24.0	170.2 ± 18.9	209.9 ± 32.3	233.2 ± 34.7	a *vs* c : p<0.01
					a *vs* d : p<0.01
					b *vs* c : p<0.01
					b *vs* d : p<0.01
					c *vs* d : p<0.01
RLP-Chol.; remnant cholesterol (mg/dL)	2.7 ± 1.2	5.0 ± 2.2	7.6 ± 9.6	5.6 ± 3.2	a *vs* c : p<0.01
DHLA, dihydrolipoic Acid(μg/mL)	33.4 ± 7.8	28.2 ± 7.0	36.5 ± 10.3	38.9 ± 12.5	b *vs* c : p<0.01
					b *vs* d : p<0.01
AA; arachidonic acid (μg/mL)	168.9 ± 37.1	159.6 ± 25.2	210.1 ± 48.4	215.5 ± 60.8	a *vs* c : p<0.01
					a *vs* d : p<0.01
					b *vs* c : p<0.01
					b *vs* d : p<0.01
EPA; eicosapentanoic acid (μg/mL)	28.3 ± 17.8	30.1 ± 16.1	87.1 ± 46.7	64.6 ± 29.0	a *vs* c : p<0.01
					a *vs* d : p<0.01
					b *vs* c : p<0.01
					b *vs* d : p<0.01
					c *vs* d : p<0.05
DHA; docosahexanoic acid (μg/mL)	79.8 ± 22.6	84.2 ± 18.0	158.6 ± 52.2	135.1 ± 28.0	a *vs* c : p<0.01
					a *vs* d : p<0.01
					b *vs* c : p<0.01
					b *vs* d : p<0.01
					c *vs* d : p<0.05
EPA/AA ratio	0.17 ± 0.11	0.19 ± 0.10	0.42 ± 0.21	0.31 ± 0.14	a *vs* c : p<0.01
					a *vs* d : p<0.01
					b *vs* c : p<0.01
blood glucose(mg/dL)	79.5 ± 12.2	68.2 ± 6.3	91.7 ± 16.3	94.5 ± 6.9	a *vs* b : p<0.01
					a *vs* c : p<0.01
					a *vs* d : p<0.01
					b *vs* c : p<0.01
					b *vs* d : p<0.01

One -way ANOVA was used for evaluating statistical significance. A,b,c,d indicate values of young and old men and women, and T2DM patients. P<0.05 is considered statistically significant.

Table 3: TRP metabolites of young and old men and women, and T2DM patients.

Sample Name	Young men n=25 a	Young women n=10 b	Old men n=20 c	Old women n=20 d	T2DM n=20 e	Significant differences
Tryptophan (μM)	63.92 ± 10.83	67.79 ± 9.30	68.89 ± 13.32	47.68 ± 7.19	64.79 ± 15.49	a *vs* d : p<0.01 b *vs* d : p<0.01 c *vs* d : p<0.01 d *vs* e : p<0.01
L-5-Hydroxytryptophan (nM)	1.32 ± 1.88	0.00 ± 0.00	0.63 ± 1.32	0.56 ± 0.95	2.19 ± 1.718	b *vs* e : p<0.01 c *vs* e : p<0.01 d *vs* e : p<0.01
Serotonin (nM)	1.556 ± 1.79	42.94 ± 35.42	30.95 ± 68.23	4.70 ± 5.86	101.22 ± 213.67	a *vs* e : p<0.01 c *vs* e : p<0.05
Kynurenine (nM)	693.9 ± 177.8	1588 ± 313	1696 ± 1074	719.9 ± 173.9	1625 ± 539	a *vs* b : p<0.01 a *vs* c : p<0.01 a *vs* e : p<0.01 b *vs* d : p<0.01 c *vs* d : p<0.01 d *vs* e : p<0.01
5-hydroxy-tryptophol (nM)	1.56 ± 5.93	0.00 ± 0.00	0.92 ± 4.07	0.13 ± 0.526	0.00 ± 0.00	-
Tryptophol (nM)	0.09 ± 0.38	4.96 ± 6.59	1.19 ± 2.95	0.10 ± 0.42	3.73 ± 16.63	-
5-Hydroxyindoleacetic acid (nM)	9.00 ± 1.92	30.87 ± 9.55	33.15 ± 12.57	13.17 ± 3.30	48.87 ± 21.18	a *vs* b : p<0.01 a *vs* c : p<0.01 a *vs* e : p<0.01 d *vs* e : p<0.01 c *vs* d : p<0.01 c *vs* e : p<0.01 b *vs* e : p<0.01
Indole-3-acetic acid (nM)	280.8 ± 161.1	2446 ± 1686	2433 ± 854	262.8 ± 142.4	3089 ± 1642	a *vs* b : p<0.01 a *vs* c : p<0.01 a *vs* e : p<0.01 b *vs* d : p<0.01 c *vs* d : p<0.01 c *vs* e : p<0.01
Anthranilic acid (nM)	3.57 ± 2.93	7.72 ± 8.592	16.04 ± 12.05	6.02 ± 6.38	21.74 ± 8.01	a *vs* c : p<0.01 a *vs* e : p<0.01 b *vs* e : p<0.01 c *vs* d : p<0.01 d *vs* e : p<0.01
Kynurenic acid (nM)	73.64 ± 23.74	53.20 ± 16.66	64.03 ± 21.65	65.40 ± 26.12	94.80 ± 34.86	b *vs* e : p<0.01 c *vs* e : p<0.01 d *vs* e : p<0.01
Quinaldic acid (nM)	5.31 ± 4.25	8.87 ± 5.12	8.82 ± 7.76	4.07 ± 2.67	9.04 ± 10.41	-
3-Indolebutyric acid (nM)	0.97 ± 1.19	15.35 ± 8.38	5.94 ± 4.78	1.24 ± 2.26	4.89 ± 7.84	a *vs* b : p<0.01 a *vs* c : p<0.01 b *vs* d : p<0.01 b *vs* e : p<0.01 b *vs* c : p<0.01 c *vs* d : p<0.01
3-Hydroxykynurenine (nM)	10.31 ± 2.24	4.42 ± 1.27	2.60 ± 2.24	12.80 ± 3.80	4.68 ± 2.35	a *vs* b : p<0.01 a *vs* c : p<0.01 a *vs* d : p<0.05 a *vs* e : p<0.01 b *vs* d : p<0.01 c *vs* d : p<0.01 d *vs* e : p<0.01
3-hydroxyanthranilic acid (nM)	4.81 ± 3.12	15.26 ± 6.08	10.34 ± 8.23	4.17 ± 4.39	20.24 ± 13.99	a *vs* b : p<0.01 a *vs* e : p<0.01 b *vs* d : p<0.01 c *vs* e : p<0.01 d *vs* e : p<0.01
Xanthurenic acid (nM)	53.05 ± 21.73	12.46 ± 7.26	12.37 ± 5.29	43.86 ± 24.64	14.87 ± 5.22	a *vs* b : p<0.01 a *vs* c : p<0.01 a *vs* e : p<0.01 b *vs* d : p<0.01 c *vs* d : p<0.01 d *vs* e : p<0.01

One -way ANOVA was used for evaluating statistical significance. A,b,c,d indicate values of young and old men and women. P<0.05 was considered significant.

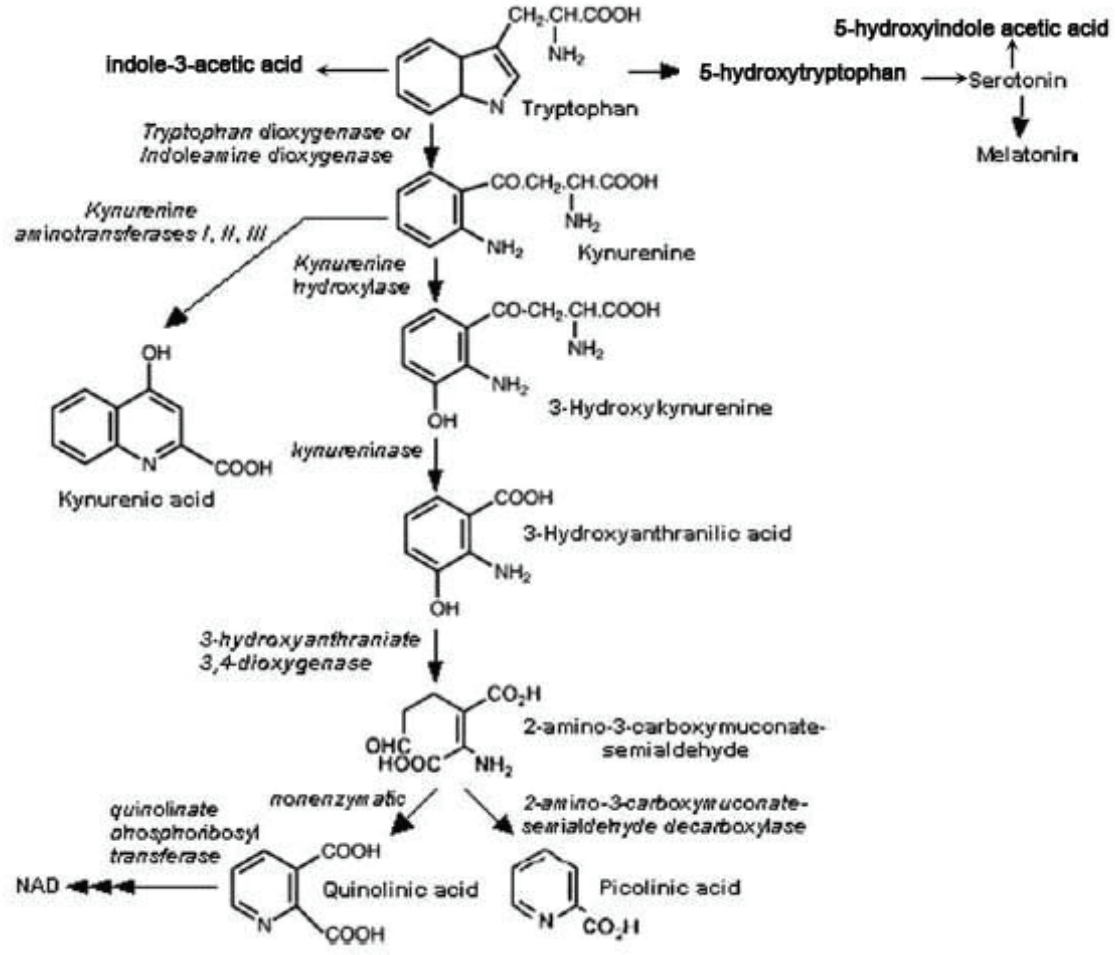

Figure 1: Shows tryptophan degradation pathway.

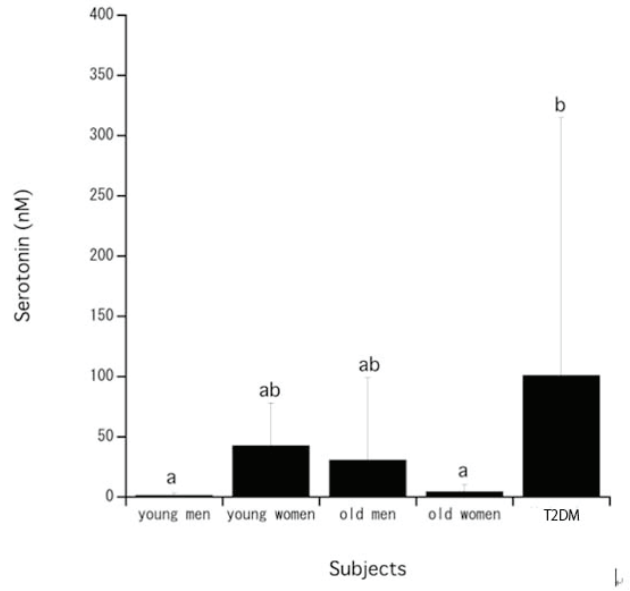

Figure 2: Serotonin levels in young and old men and women, and T2DM patients.

a vs b; $p<0.05$, ab vs a or b; not significant

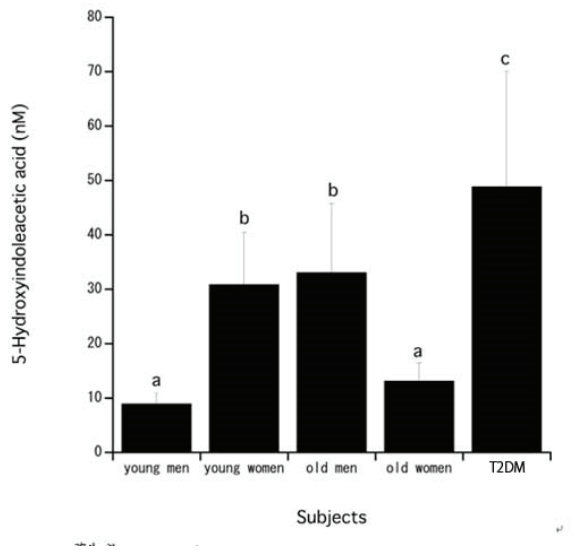

Figure 3: 5-HIAA (5-hydroxyindoleacetic acid) levels in young and old men and women, and T2DM patients. It shows that 5-HIAA levels were higher in old men than T2DM patients.

a vs b or c; $p<0.05$

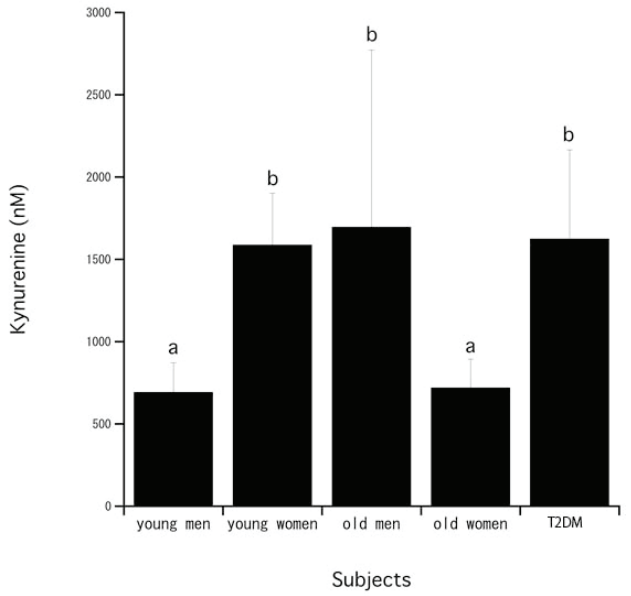

Figure 4: Plasma levels of KNY in old and young men and women, or T2DM patients. It shows that there was no statistical significance in KNY levels between old men and T2DM patients.

a vs b; $p<0.05$

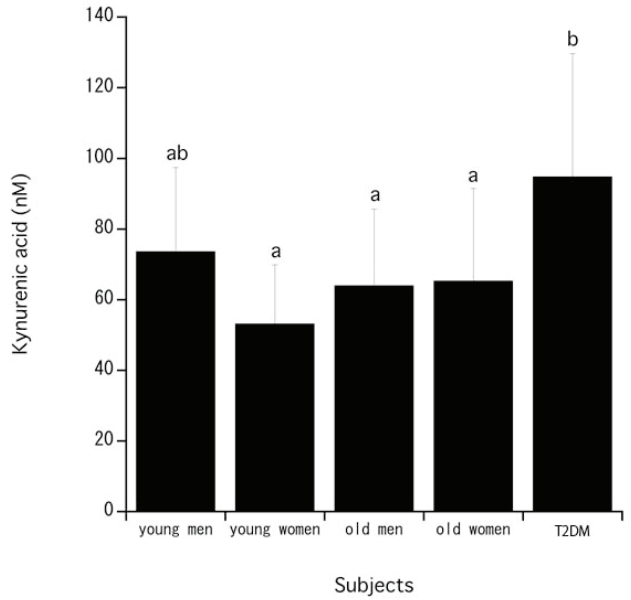

Figure 5: Plasma levels of KYNA in young and old men and women, and T2DM patients.

a vs b; $p<0.05$.

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